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Oxygen isotope ratios in mosses from the Lake Superior region: implications for paleoclimatic studies

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Oxygen isotope ratios in mosses from the Lake Superior region: implications for paleoclimatic studies

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ABSTRACT

Sedimentological studies of beach ridge sequences that are commonly present along the coastline of Lake Superior indicate large and relatively rapid lake level fluctuations over the past 4,700 years, which resulted from the interplay of climate, differential isostatic rebound, and outlet dynamics. The presence of peat deposits in swales between the beach ridges offers an opportunity to assess the role of climate because the isotopic composition of cellulose extracted from peat can be used to assess changes in water balance, moisture sources, and/or local hydrological conditions. However, despite its great potential as a paleoclimate proxy, the factors that control the isotopic composition of cellulose in modern plants are still uncertain.

To clarify the relationship between climate and cellulose isotopic compositions, samples of mosses, pond water, and groundwater were collected in three locations (Au Train, Grand Traverse, and Ontonagon) along the southern shoreline of Lake Superior throughout the 2003 and 2004 growing seasons. These three sites were selected because they represent the same sequences of swales and beach ridges that have been used to reconstruct water levels in Lake Superior (Johnston et al., 2003).

Isotope data of water samples show that the studied ponds experience different hydrology. Whereas groundwater inflow dominates the hydrological balance of the ponds located in Grand Traverse and Ontonagon, groundwater and meteoric precipitation equally influence the hydrology of the pond located in Au Train. None of the investigated ponds shows clear evidence of evaporative enrichment.

Isotopic results of the *Sphagnum* samples collected in Au Train exhibit a small variability of cellulose $\delta^{18}O$ values compared to pond water $\delta^{18}O$ values. Whereas the range in pond

water $\delta^{18}O$ values is 4.4‰ (from –11.14‰ to –6.76‰), moss cellulose exhibits a range of only 2.2‰ (from 17.2‰ to 19.42‰). Given the fairly invariable ecology and phenology of the sampled mosses, it is concluded that the reduced variability in cellulose $\delta^{18}O$ values is caused by the continuous, water-unlimited growth of mosses throughout the entire growing seasons. In fact, moss cellulose at Au Train reflects the average isotopic composition of pond water during the growing season.

Isotope data for the moss samples collected in Grand Traverse and Ontonagon show a higher variability in cellulose $\delta^{18}O$ values relative to the Au Train samples. In addition, it was found that ecology and phenology affect the cellulose $\delta^{18}O$ values of the studied samples. For example, at Grand Traverse, the average cellulose $\delta^{18}O$ value of *Sphagnum cuspidatum* (16.4‰) is 3.2‰ lower than the average cellulose $\delta^{18}O$ value of *Drepanocladus fluitans* (19.62‰). Evaporation of leaf water likely causes the enrichment of ^{18}O in *Drepanocladus fluitans* with respect to *Sphagnum cuspidatum*-cellulose.

Because of the presence of species with different ecology in the three sites and because of the different hydrology of the ponds, it is concluded that any peat-based paleoclimatic investigation of the area has to be strictly site-specific and has to be conducted at the species level.

INTRODUCTION

A lake is the landscape's most beautiful
and expressive feature. It is earth's eye;
looking into which the beholder measures
the depth of his own nature.
Thoreau

Covering an area of about 82300 km^2, Lake Superior is the largest freshwater lake in the world. It lies across the international border between the United States and Canada. The Canadian province of Ontario is to the north and east of the lake, Michigan and Wisconsin lie to the south, and Minnesota to the west. It is about 400 meters deep at its deepest point and holding over 14000 km^3 of water it provides a very important resource of water for consumption, transportation, power, and recreation.

Water levels of Lake Superior and of the other Great Lakes have changed throughout the late Holocene as shown by Thompson (1992). This author showed the existence of three cycles of quasi-periodic lake level variations occurring every 25-35, 140-160, and 500-600 years. These fluctuations can severely affect coastal wetlands, surrounding flora and fauna, water quality, and the economy of the region (Keddy and Reznicek, 1986; Wilcox, 1989). Documenting the magnitude and frequency of past Great Lakes water level fluctuations and understanding their causes is, therefore, very important in order to optimize water resource management, to help understand past climate changes, and to predict potential future changes in lake levels.

Several authors have invoked climate as the forcing mechanism that causes these fluctuations (e.g., Quinn, 2002; Argyilan and Forman, 2003). This suggestion is based on

gauge records that show a strong correspondence between climate and lake levels (Croley et al., 1996) (Figure 1), indicating that whereas droughts in the region cause lake level falls, increased rainfall produces lake level rises. Although gauge records are a useful source of information, they are available only for the last 140 years, which is less than 5% of the time period during which the Great Lakes basin has been in existence with its current drainage basin. A more accurate investigation of the causal relationship between climate and lake levels requires, therefore, a longer-term reconstruction of the climate history of the region.

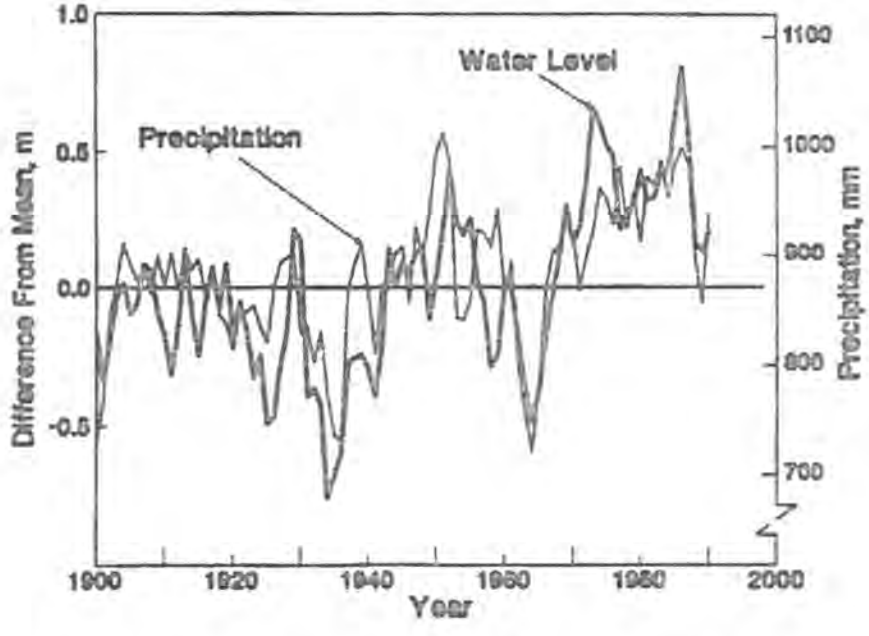

Figure 1. Lake Erie annual water levels and precipitation (Croley et al., 1996).

Several studies investigating the Holocene climate of the Great Lakes region have revealed that climate has changed with a relatively high frequency. For example, Davis et al. (2000) reconstructed temperature and rainfall over the past 10000 years using pollen records from deposits collected in six different national parks and lakeshores of the western Great Lakes region. They found that in the early Holocene the region experienced colder winters,

warmer summers, and lower precipitation relative to modern conditions. While a warm and dry event prevailed in the region during the mid-Holocene, increased rainfall dominated the late Holocene. These characteristics represent the broad trend of the Holocene climate history of the region as indicated by a number of studies (e.g., Yu et al., 1997; Delcourt et al., 2002) that confirmed the existence of a warm and dry period during the mid-Holocene. Delcourt et al. (2002), studying the carbon and oxygen isotopic composition of sediments from Nelson Lake on Michigan's Upper Peninsula, dated the occurrence of this mid-Holocene dry period between 8000 and 5300 years B.P.. They also found a significant trend toward a cooler and more humid climate occurring between 5300 and 3000 years B.P.

In a recent study, Booth et al. (2002) investigated the relationship between these climatic changes and fluctuations in water levels of the Great Lakes by analyzing pollen and macrofossil assemblages from a sediment core extracted from a small lake located in Michigan's Upper Peninsula. These authors found a good correspondence between highstands in water levels (called Algoma, Nipissing I and II phases) and increased precipitation. According to these authors, the Algoma and Nipissing II phases, occurring between 4600 and 4400 years B.P. and around 3200 years B.P. respectively, were the result of increased moisture balance throughout the entire region. The occurrence of the Nipissing I phase (between 6800 and 5700 years B.P.), on the other hand, is explained as the result of an antipodal effective moisture change in southern and northern portions of the western Great Lakes region. Thus, the high water levels of the Nipissing I highstand were produced by climatic changes that occurred in the northern part of the Great Lakes basin since this part experienced an increase in effective moisture. Because most of the western Great Lakes catchment is in the northern part of the basin, lake levels are more sensitive to climate

changes affecting that portion of the basin. The result of Booth et al.'s study thus support the notion that climate is the main factor driving water level fluctuations in the Great Lakes.

Other mechanisms, however, have been proposed to explain the fluctuations in water levels (Larsen, 1994; Baedke and Thompson, 2000). These mechanisms include glacioeustatic rebound and outlet dynamics. The first process results from uplifting of the Earth's crust following the melting of the ice sheet that covered most of North America during the Pleistocene. In fact, isostatic rebound is still causing the northeastern part of the Lake Superior basin to rise relative to the southwestern part (Ferrand and Drexler, 1985). This process influences the water level of the Great Lakes by changing the river slopes and by tilting the lake basin (Balco et al., 1997). The second process refers to the change in the elevation and/or position of the main outlet(s) that happened repeatedly throughout the history of the lakes. This process results from post-glacial rebound or from the erosion caused by discharge streams (Dorr and Eschman, 1970).

Even if climate is considered the main factor triggering water level fluctuations in the Great Lakes, the mechanism responsible for changing the water balance in this region is still under debate. For instance, Dalrymple and Carey (1990) suggest that water level oscillations in Lake Ontario correlate with climate changes recorded in northeastern North America, rather than with climate changes recorded elsewhere in the Great Lakes basin or in the Midwest. In contrast, Fraser et al. (1990) propose a local effect in which high lake levels occur when there is a convergence of Arctic and tropical air masses over the Great Lakes basin, leading to increased winter precipitation in its watershed and the consequent increase of melt water runoff to the lakes during the spring. Low lake levels are then postulated to

occur when air masses from the Pacific prevail over the Great Lakes, reducing precipitation and increasing evaporation.

In addition to these uncertainties, predictions of the effects of future climate changes on the Great Lakes are contrasting. A current concern is that global warming resulting from an increase in the concentration of atmospheric CO_2 and other greenhouse gases could produce major changes to the hydrologic cycle (Quinn, 1988). Estimates based on a doubling of atmospheric CO_2 concentrations indicate a temperature rise of about 4°C over the latitudinal range of the Great Lakes basin (Cohen, 1986). In addition, changes in the amount and seasonal distribution of precipitation and major changes in wind velocities would likely occur (Quinn, 1988). Some experiments using General Circulation Models (GCM) predict a 20% reduction in rainfall and a 0.2-0.5 m decrease in water levels (Mortsch and Quinn, 1996). Other simulations predict an increase in lake levels induced by elevated precipitation rates (Lofgren et al., 2002). The reason for these contrasting results is a poor understanding of the relationship between climate and hydrology in the Great Lakes region. To resolve these contrasting predictions, it is necessary to understand better the linkage between climate and hydrology to improve climate predictions and reduce the impact of future changes in water levels for the Great Lakes region.

A comprehensive assessment of the impact of climate on water levels requires accurate reconstructions of Holocene water levels of the Great Lakes, which can be achieved by studying the sequences of beach ridges commonly present along the coastline of the Great Lakes. Thompson (1992) reconstructed water levels of Lake Michigan for the last 4000 years by analyzing vibracores of beach ridges and intervening wetlands present in the southern margin of the lake. His results indicate that beach ridges are composed of a core of foreshore

(i.e., swash zone) sediments that are capped by dune deposits and underlain by upper shoreface sediments. The elevation of the coarse-grained base of the foreshore sequence is a close approximation of the lake level when the beach ridge formed (Thompson et al., 1988) (Figure 2). A temporal framework for the development of these beach ridges can be obtained from radiocarbon dates of peat deposits found among the ridges, thereby allowing a reconstruction of the history of lake levels during the late Holocene. The main assumption behind this type of studies is that the wetlands found in swales between the beach ridges developed soon after the formation of the ridges. This assumption is valid if the wetlands formed and began accumulating organic sediment 40 to120 years after the development of the beach ridges because this time interval represents the standard laboratory error on most

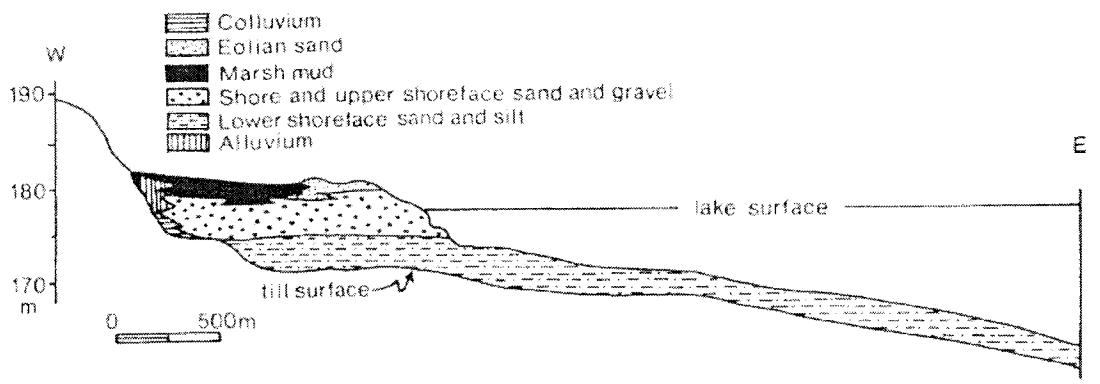

Figure 2. Schematic cross section of a beach ridge, indicating that the elevation of shoreface deposits approximately coincides with lake levels (Fraser et al., 1990).

radiocarbon-date determinations (Thompson, 1992). Using this approach, Johnston et al. (2003) reconstructed water levels for Lake Superior from 4200 to 2100 cal. years. B.P.. Their results indicate that lake levels dropped rapidly from 4100 to 3800 cal. years. B.P., lowered gradually from 3800 to 2400 cal. years B.P., and remained constant from 2300 to 2100 cal. years. B.P..

The presence of peat deposits in swales between beach ridges also offers an opportunity to assess the role of climate because stable isotope ratios of cellulose extracted from peat have been used to reconstruct changes in atmospheric patterns (e.g., Edwards et al., 1996). Climate patterns in the region are determined by the relative predominance of air masses from the Arctic, from the Gulf of Mexico, and from the Pacific Ocean (Bryson and Hare, 1974) (Figure 3). During boreal winter, the climate of the region is dominated by a persistent northwesterly flow of Pacific airstream. The airstream loses its initial moisture over the Rocky Mountains, thereby becoming a cold, dry, and stable continental air mass. Strong net outward radiation from snow above the Arctic Circle converts invading air into Arctic air masses, which generally form a cold anticyclone and cause polar outbreaks (Bryson and Hare, 1974). Incursions of Pacific air are less common in the spring and the outbreaks of

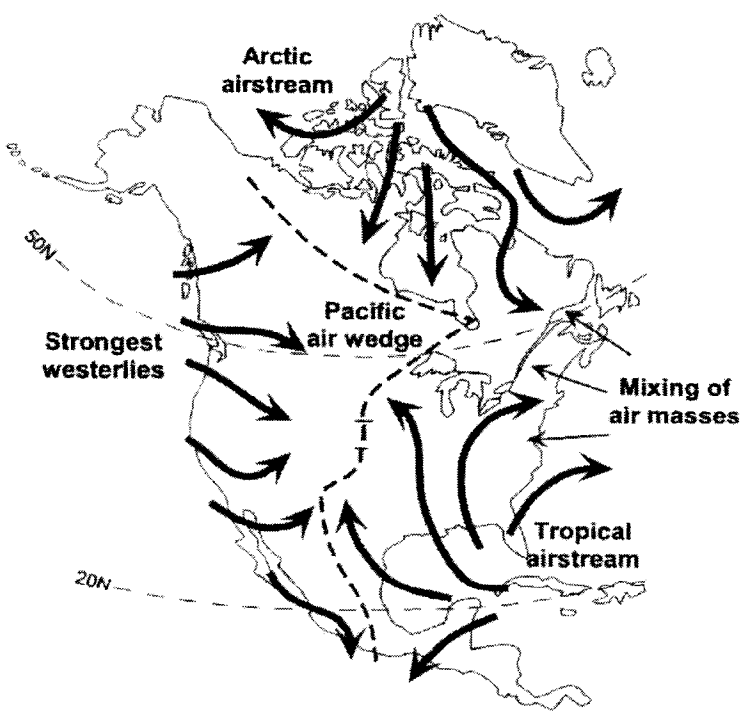

Figure 3. The different air masses influencing the weather in the Great Lakes region (modified from Simpkins, 1995).

Arctic air are not found as far south as in the winter months (Simpkins, 1995). During boreal summer, a weakening and retreat of the Arctic air is observed, and the weather is dominated by an interaction of Pacific air and air masses from the Gulf of Mexico which produces violent thunderstorms. When Gulf air moves over the warm continent, the temperature of the air mass increases, creating atmospheric instability, which, coupled with a high relative humidity of the air, produces abundant showers and thunderstorms. During boreal autumn, the weather is marked by the reoccurrence and strengthening of the westerly Pacific winds (Simpkins, 1995). Air masses originating in the Gulf of Mexico represent, therefore, the main source of moisture of summer rainfall. In contrast, the relative contribution of moisture associated with air masses from the Pacific increases in spring and winter (Bryson and Hare, 1974).

Moisture associated with these different air masses can be distinguished on the basis of their different isotopic signature. Moisture originating from the Gulf of Mexico is more enriched in the heavier isotopes (^{18}O and D) than moisture coming from the Pacific and from the Arctic (Simpkins, 1995). This is because, in a humid and warm environment such as that of the Gulf, abundant energy derived from warm waters and a reduced vapor gradient favor the incorporation of the heavier isotopes in the vapor phase. In contrast, a drier and colder environment, such as that of the Pacific Ocean, enhances the fractionation of the heavier isotopes during the evaporation of oceanic water (Dansgaard, 1964). In addition to this fractionation effects associated with evaporation, air masses from the Pacific are subjected to a progressive depletion in the content of ^{18}O and D as they move inland ("continental effect", Dansgaard, 1964) and cross the Rocky Mountains ("altitudinal effect", Dansgaard, 1964). Since photosynthetic organisms in lakes and ponds utilize surrounding water to synthesize

cellulose, it could be possible to distinguish periods of different relative predominance of these two air masses by analyzing the isotopic composition of the peat cellulose.

Several authors have used the isotopic composition of cellulose to assess changes in water balance, moisture sources, and/or local hydrological conditions (Aucour et al., 1996; Edwards et al., 1996; Beuning et al., 1997; Wolfe and Edwards, 1997; Wolfe et al., 2000). These studies rely on temperature-dependent isotopic signals inherited by cellulose from local meteoric water. This temperature-dependent signal derives from the fact that condensation, which favors the incorporation of the heavy isotopes in the condensate, increases as temperature decreases (Bradley, 1999). For instance, the study of Wolfe and Edwards (1997) highlights the possibility of inferring lake-specific hydrologic information by studying the isotopic composition of cellulose disseminated in lacustrine organic matter. Based on the difference between observed and calculated cellulose-water relationships, they estimated the importance of different sources for the water balance of several tundra and forest lakes of northern Russia. They found that the tundra lakes were more sensitive to snowmelt contributions than forest lakes. Another example is provided by Wolfe et al. (2000) who reconstructed the Holocene paleohydrology of two regions along the boreal tree-line in western Siberia. Using data obtained from cellulose and from paleowaters preserved in frozen peat deposits, they found a strong longitudinal moisture gradient in the Russian arctic at about 9000 ^{14}C years B.P.. This gradient, which ceased 3500 ^{14}C years ago, was caused by the development of a more maritime climate in western Russia with respect to the eastern part of the country. The inferred paleoclimatic conditions seem to coincide with tree-line migrations investigated by MacDonald et al. (2000).

Despite its great potential as a climate archive, the application of peat cellulose as a paleoclimate proxy is still uncertain. Only a handful of studies have focused on the relationship between climate and cellulose isotope ratios in modern peatlands. Moreover, these studies (e.g., Aravena and Warner, 1992; Menot-Combes et al., 2002) are restricted to ombrogenic bogs (i.e., wetlands in which meteoric precipitation is the only form of water supply). Therefore, the main purpose of this study is to analyze the isotopic composition of modern mosses collected in fens (i.e., wetlands considerably influenced by groundwater) along the shoreline of Lake Superior to calibrate plant-climate relationships. To do that, it is necessary to investigate the relationship between the isotopic composition of moss cellulose and the isotopic composition of the possible water sources of the mosses, namely rainwater, pond water, and groundwater. Since *Sphagnum* mosses are the main constituents of peat deposits in the Great Lakes region (Crum, 1988), these relationships could be used to improve the paleoclimatic interpretation of peat cellulose isotope data and to investigate whether variations in Holocene water levels of the Great Lakes can be correlated to variations in atmospheric circulation patterns over the region.

STABLE ISOTOPES

Basic concepts

Isotopes are atoms with the same number of protons and electrons but with a different number of neutrons. In contrast to radioisotopes, which spontaneously decay and transform into other nuclides emitting alpha or beta particles and sometimes gamma rays during the disintegration of their nuclei, stable isotopes do not undergo nuclear transformations over time. This study focuses on the variability of oxygen and hydrogen isotopes in moss cellulose

and its potential water sources. Oxygen has three naturally occurring stable isotopes whose atomic masses are 16, 17, and 18 (designated ^{16}O, ^{17}O, and ^{18}O), whereas hydrogen has two stable isotopes whose masses are 1 and 2 (H, protium, and D, deuterium, respectively). Average terrestrial abundances of ^{16}O, ^{17}O, and ^{18}O are 99.76%, 0.035%, and 0.2%, respectively. Average terrestrial abundances of H and D are 99.985% and 0.015%. Because of the existence of different isotopes of hydrogen and oxygen, nine isotopically different water molecules can theoretically be present in nature. However, considering the abundance of the isotopes, only three ($H_2{}^{16}O$, $H_2{}^{18}O$, $HD^{16}O$) occur in detectable concentrations. Hence, this study will only discuss the variability of the ratios $^{18}O/^{16}O$ and D/H.

Molecules containing different isotopes of the same element have different physical and chemical properties because of differences in the mass of the atomic nuclei. The consequences are two-fold (Mook and de Vries, 2000):

- The heavier isotopic molecules have a lower mobility, and
- The heavier isotopic molecules generally have higher binding energies.

Because of these differences between molecules containing heavy and light isotopes, fractionation of isotopes occur during certain chemical reactions or phases transitions. These fractionation processes involve the partitioning of isotopes among two substances or among different physical phases of the same substance. For instance, $H_2{}^{18}O$ and $HD^{16}O$, having lower vapor pressure than $H_2{}^{16}O$, will evaporate less readily and will be preferentially incorporated in the phase with the larger molecular weight during liquid-gas phase transitions.

It is also important to distinguish between two kinds of isotope fractionation: kinetic fractionation and equilibrium fractionation. The former results from irreversible (i.e., one-

way) physical or chemical processes; the latter is essentially the isotope effect involved in a thermodynamic equilibrium reaction. For both processes, the extent of fractionation is described by the fractionation factor ($\alpha_{A\text{-}B}$) which is defined according to this relation:

$$\alpha_{A\text{-}B} = R_A/R_B \quad (1)$$

R refers to the atomic ratio of the heavy (in our case ^{18}O or D) to the light (^{16}O or H) isotopes and A and B can be either two different substances or the same substance (e.g., water) but in different physical phases. Since in general $\alpha \approx 1$, the deviation from 1 is often used (the enrichment or depletion factor):

$$\varepsilon_{A/B} = (\alpha_{A\text{-}B} - 1) \cdot 10^3 \text{ (‰)} \quad (2)$$

The enrichment or depletion factor is commonly expressed as parts per thousand or "per mil" (‰).

The isotopic composition is customarily expressed in terms of the conventional delta notation, where the isotopic composition of the studied substrate is reported relative to that of an international standard as indicated by the following expression:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 10^3 \text{ (‰)} \quad (3)$$

where R is the ratio of heavy to light isotope forms ($^{18}O/^{16}O$ or D/H in our case). δ values are expressed as parts per thousand or "per mil" deviation from the standard. The standard for both hydrogen and oxygen is V-SMOW (Vienna Standard Mean Ocean Water) with an absolute isotope ratio for $^{18}O/^{16}O$ of:

$$R = 2.0052 \cdot 10^{-3} \quad (4)$$

Stable isotope ratios of plant cellulose

Cellulose is the basic structural component of plant cell walls and is the most abundant of all naturally occurring organic compounds. Cellulose is a polymer consisting of linear chains of anhydroglucose units (Figure 4). A polymer is simply a larger molecule consisting of many smaller, repeated subunits. In the case of cellulose, the subunit is glucose. The structure of cellulose consists, therefore, of repeated units of $C_6H_{12}O_6$, or glucose (Green, 1963).

In general, the oxygen and hydrogen isotopic composition of plant cellulose is determined by (Brenninkmeijer et al., 1982):

- The isotopic composition of the source water (i.e., water used by the photosynthetic organisms to synthesize cellulose),
- The leaf water enrichment (i.e., a process taking place in the leaves of the plant caused by evapotranspiration that leads to an increase in the δ values of leaf water), and
- The biochemical fractionation (i.e., a series of fractionation processes that accompany the biochemical reactions in plants).

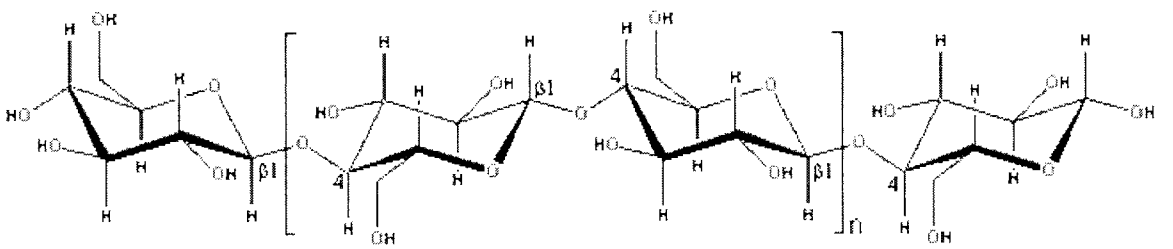

Figure 4. Structural units in cellulose.

Models developed to calculate the oxygen and hydrogen isotopic composition of cellulose (δ_{cell}) include these three components (Waterhouse et al., 2002):

$$\delta_{cell} = \delta_S + \varepsilon_b + (\varepsilon_e + \varepsilon_K) \cdot (1 - h) \quad (5)$$

where δ_S is the isotopic composition of the source water, ε_b is the biochemical enrichment effect, ε_e is the liquid-vapor equilibrium effect associated to the leaf water enrichment, ε_K is the liquid-vapor kinetic enrichment effect occurring during evaporation, and h is the relative humidity. Because the studied mosses are aquatic plants, h is close to 1 in eq. 5. Consequently, the isotopic composition of moss cellulose can be expressed by the following equation:

$$\delta_{cell} = \delta_S + \varepsilon_b \quad (6)$$

Source water

In the case of aquatic plants growing in lakes and ponds, source water is represented by lake water or pond water. The isotopic composition of lake or pond water, in turn, is determined by the isotopic composition of local meteoric water and by secondary factors such as evaporation, snowmelt mixing (for high altitude and high latitude lakes), and groundwater inflow.

The isotopic composition of meteoric water depends mainly on the temperature of condensation, the source of water vapor, and the distance from that source. In general, the following effects can be observed (Mook and de Vries, 2000):

- Latitudinal effect: lower δ-values at increasing latitude.
- Continental effect: lower δ-values at increasing distance from the source of the moisture.
- Altitude effect: lower δ-values at higher altitude.

- Seasonal effect (in regions with temperate climate): lower δ-values during winter.
- Amount effect: lower δ-values during heavy storms.

In addition, modern meteoric waters at a global scale closely conform to an empirical relationship known as the "global meteoric water line", or GMWL (Craig, 1961) (Figure 5):

$$\delta D = 8\delta^{18}O + 10 \quad (7)$$

δD and $\delta^{18}O$ values of precipitation falling at individual sites over a year commonly exhibit a strong linear correlation between their δD and $\delta^{18}O$ values, defining lines termed "local meteoric water lines" (LMWLs) (Craig, 1961).

Evaporation is the most prominent example of non-equilibrium fractionation process operating in the water cycle. This process is more pronounced for water bodies in windy, hot, and arid areas (Gat and Gonfiantini, 1981). Evaporation discriminates between different isotopes by allowing the lighter isotope to be preferentially incorporated in the vapor phase, which produces a relative enrichment of the heavier isotope in the liquid phase. Deuterium and ^{18}O are enriched in such a way that lake water oxygen and hydrogen isotopic composition deviates from the meteoric water line, plotting along lines with a smaller slope than that of the GMWL. These lines are termed "local evaporation lines" (LELs, Gonfiantini 1986). Intersection of the LEL with the LMWL often provides an excellent approximation of the weighted-mean isotopic composition of rainwater in a catchment (Edwards et al., 2004) (Figure 5). An example of evaporative enrichment is provided by Menot-Combes et al. (2002) who investigated the isotopic composition of water samples collected in 13 bogs in Switzerland. The δD and $\delta^{18}O$ values of water samples plotted along well defined LELs. These authors also showed that evaporation leads to variations in the isotopic composition

of bog water that are related to the microtopography of the bog surface. They observed higher δ–values in water samples collected from small raised hummocks than those collected

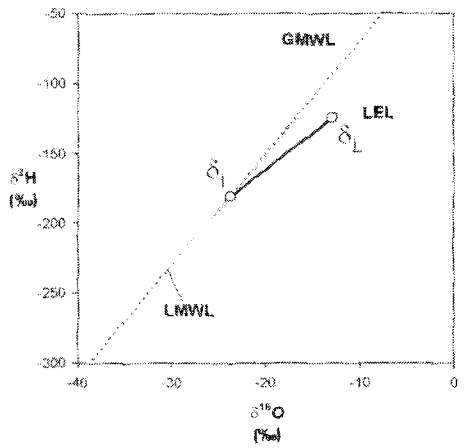

Figure 5. Isotope labeling of water balance components for a hypothetical middle latitude lake. The isotopic composition of the lake water at the time of sampling (δ_L) is offset from the input water composition (δ_I) along a local evaporation line (LEL) having a smaller slope than the LMWL (modified from Edwards et al., 2004).

from hollows in both horizontal and vertical profiles.

Groundwater inflow can alter the isotopic composition of lake water because most groundwater bodies are isotopically constant and closely reflect the average annual isotopic composition of local precipitation (Gat and Gonfiantini, 1981). This means that the seasonal variability in isotopic composition exhibited by surface water is considerably dampened or even absent in groundwater-dominated systems. A good example of isotopic analysis of lake water focusing on groundwater inflow is provided by Hammarlund et al. (2002). These authors showed that the isotopic composition of Lake Tibetanus (Sweden), a particular water body characterized by complete absence of evaporative enrichment, is associated with rapid throughflow of local groundwater. The δD and $\delta^{18}O$ values of lake water plotted along the

LMWL and showed a small variability compared to the isotopic composition of local rainwater.

Snowmelt mixing is another factor influencing the isotopic composition of lake water. Because winter precipitation is depleted in the heavier isotopes relative to rainwater falling in other seasons ("seasonal effect"), appreciable snowmelt content in lake water can cause lower $\delta^{18}O$ and δD values. The effect of snowmelt dilution is clearly visible in the data obtained by Maric (2003) who studied the seasonal variability of 30 small tundra lakes in the West Exeter Lake catchment, Canada. During spring melt (mid-June 2001), δ-values for these lakes varied from –23 to –18‰ for oxygen and from –180‰ to –160‰ for hydrogen. Two months later, the same lakes showed values ranging from –17‰ to –14‰ for oxygen and –160‰ to –140‰ for hydrogen. This study provides a typical example of the contrasting isotopic effect of snowmelt dilution and evaporative enrichment on high latitude lake waters.

Leaf water enrichment

Although there is no fractionation against oxygen isotopes during water uptake by roots (Zimmermann et al., 1967; White et al., 1985), water in the leaves is enriched in ^{18}O because of evaporation. The classical model for evaporative enrichment was developed by Craig and Gordon (1965). This model has the following form:

$$\delta_E \cong \frac{\delta_L - h^* \cdot \delta_a - \varepsilon_{eq} - (1 - h^*) \cdot \varepsilon_K}{1 - h^*} \qquad (8)$$

where δ_E, δ_L, and δ_a are the isotopic compositions of evaporating water, liquid water, and ambient air moisture, respectively; ε_{eq} and ε_K are the equilibrium and kinetic enrichment factors for the liquid-vapor phase transition, and h* is the relative humidity of ambient air at

surface temperature. This model provides a quantitative assessment of ^{18}O enrichment of liquid water left behind after the evaporation of ^{18}O-depleted vapor from the leaf surface.

Flanagan et al. (1991) expanded the Craig-Gordon model to include leaf boundary layer considerations and diffusion through stomata, making it more appropriate for modelling leaf water:

$$R_{wl} = \alpha^* \left[\alpha_k - R_{wx} \left(\frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_{wx} \left(\frac{e_s - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right] \quad (9)$$

where R are the isotope ratios, the subscripts wl, a, i, s and wx refer to leaf water, bulk air, intercellular air spaces, leaf surface, and xylem water, respectively. α^* is the temperature-dependent liquid-vapor equilibrium fractionation factor, α_k is the kinetic fractionation associated with diffusion in air, α_{kb} is the kinetic fractionation associated with the boundary layer, e_i is the vapor pressure of the intercellular air spaces of the leaf, e_s is the vapor pressure at the leaf surface (which depends on the physiologic characteristics of the leaf), and e_a is the vapor pressure of bulk air. This equation predicts isotopic values of water at the site of evaporation. Further fractionation, called biochemical fractionation, occurs within the chloroplasts.

Biochemical fractionation

The biochemical pathway leading to cellulose synthesis is accurately described in a review published by Farquhar et al. (1998). The pathway starts with the Calvin and photorespiratory cycle and continues with the synthesis of sucrose, starch, and finally cellulose. In C3 plants, the photosynthetic pathway used by mosses (Proctor et al., 1992), CO_2 enters the leaf through the stomata, diffusing into the mesophyll cells where the enzyme Rubisco catalyzes the carboxylation (addition of CO_2) of RuBP (Ribulose bisphosphate) to

form two PGA (phosphoglycerate) molecules (Ehleringer and Monson, 1993). These molecules are subsequently converted into glyceraldehyde 3-phosphate, which is then transformed into fructose, glucose, and sucrose. Sucrose is the major carbohydrate that is transported throughout the plant and is the substrate used to synthesize cellulose. The reactions involved in the biosynthesis of cellulose are complex and considerably numerous. Each of these reactions can, in theory, be accompanied by an isotopic fractionation process. However, according to Yakir and DeNiro (1990), the oxygen isotopic composition of cellulose is determined by a single type of exchange reaction with water, which explains the commonly observed enrichment of 27±3‰ of cellulose relative to water at the site of synthesis (Epstein et al., 1977; De Niro and Epstein, 1981; Aucour et al., 1996). This isotope equilibration effect takes place during the hydration of carbonyl groups, functional groups that consist of an oxygen atom joined to a carbon atom by a double bond. This hydration reaction occurs throughout the entire biosynthesis of carbohydrates and it is likely to be insensitive to temperature (Wolfe et al., 2001).

Another model proposed to explain the enrichment of cellulose relative to its source water is given by Epstein et al. (1977). These authors suggested that one-third of the oxygen atoms in cellulose come from the source water and two-thirds come from the equilibrated dissolved CO_2. Dissolved CO_2 is enriched by 41‰ relative to the source water at 25°C, given that the oxygen isotope fractionation factor between CO_2 and H_2O at air temperature is 1.0412. Therefore, the incorporation of two-thirds of the oxygen from the dissolved CO_2 produces the value of the enrichment factor of 27‰ (i.e., $2/3 \cdot 41 \approx 27$‰). However, as discussed by Yakir (1992), there are several difficulties with this theory. For instance, this isotopic relationship exists in plants that do not consume CO_2 (heterotrophic metabolism).

Moreover, the isotopic effect associated with CO_2 equilibration with water is highly sensitive to temperature, but no clear temperature-dependent isotope effect has been demonstrated between water and cellulose.

MOSSES

Mosses, along with liverworts and hornworts, belong to a division of the plant kingdom known as Bryophyta. Morphologically, bryophytes are usually small organisms, typically green, and lacking some of the complex structures found in vascular plants. They do not produce flowers or seeds, and the majority have no internal structures for transporting water or nutrients. Although they have no roots, they have root-like structures for anchoring and water absorption called rhizoids (Hallingback and Hodgetts, 2000).

The life cycle of mosses consists of two distinct phases, sexual and asexual. Each phase produces the other, and this process is referred to as the alternation of generations. The sexual phase of the plant begins its growth from an asexual spore. The spore produces a small, threadlike, and trailing structure called protonema, which branches and spreads over the ground. Minute buds form on the branches and develop into leafy plants that are commonly recognized as mosses. At the tips of the leafy shoots, entirely hidden by the leaves, are the reproductive or sex organs. The male reproductive organ is called antheridium, and the female organ is called archegonium. Sex cells produced by the reproductive organs are known as gametes and the moss plant in this stage is called a gamethotype. During this stage, the male sperm cell, carried by water, enters the archegonium and unites with the egg cell it contains. This union is known as fertilization, and it represents the beginning of the next stage of the moss reproductive cycle. During this

stage, called sporophyte, the moss plant is no longer a producer of gametes but a producer of spores. The sporophyte grows directly out of the leafy female gamethotype. As it becomes larger, it forms a leafless stalk, or seta, that connects it to the female plant. At the tip of the seta, a kind of capsule or case develops. This is the part of the sporophyte that will produce spores. As the sporophyte develops, thousands of tiny spores form within its capsule. When the spores become ripe, a small cap located at the tip of the capsule, called calyptra, fall off. The spores are then released and, if they fall where conditions are favorable, a new generation of gamethotypes will grow (Johnson, 1983).

The mosses studied in this work belong to five different genera: *Sphagnum*, *Drepanocladus, Aulacomnium*, *Calliergon*, and *Callicladium*. The genus *Sphagnum* is commonly known as peat moss. The peat mosses differ morphologically from other mosses in the way their branches are organized on the plant. Branches are arranged in clusters called fascicles along the erect stem. Each fascicle consists of two or more spreading branches and one or more pendent branches. Young branches are usually crowded together at the top of the plant to form a head or capitulum. Along the branches, there are leaves (called branch leaves) arranged loosely or organized in five distinct rows. Another kind of leaves, called stem leaves, is found along the main stem. Stem leaves and the number and kind of branches in the fascicles are important clues in species identification (McQueen, 1990).

The species studied in this work include *Sphagnum cuspidatum*, *Sphagnum centrale*, and *Sphagnum squarrosum. Sphagnum cuspidatum* is characterized by large, slender, bright green to yellow plants with a wet feathery appearance. The branches in the capitulum may have slight reddish tinge at the base. The capitulum is not well developed, it is not very noticeable, and the terminal bud is small. Branch leaves are very long (1.5-3 mm or more),

lanceolate, and curved, especially near the tips of the branches where sometimes almost all the leaves curve in the same direction. Stem leaves are smaller than branch leaves (1-1.3 mm), triangular, with an acute or slightly rounded apex and with small teeth. This species is primarily aquatic, it often grows submerged or near the water level at the edges of floating mats and in shallow pools (McQueen, 1990).

Sphagnum centrale is characterized by large, robust, pale green to green plants. The capitulum is composed of short, stout branches. Branch leaves are very large (1.5-2.5 mm long), ovate, and very concave. Small teeth are present near the apex on the back side of the leaf. Stem leaves are fan-shaped, widest above the middle, and characterized by a broadly rounded apex with a fine fringe sometimes extending down the sides of the leaf. It usually grows in shaded habitats forming carpets surrounding open peatlands (McQueen, 1990).

Sphagnum squarrosum are tall, robust, stiff, pale to bright green plants. It has a prominent terminal bud surrounded by very short, loosely packed branches. Branch leaves are large (0.9-2.5 mm long), definitely squarrose, with the apex bent from 45° to 90° from the branch axis. The lower half of the leaf is nearly oval, tapering sharply to the tip. Stem leaves are large (1.5-2.5 mm long), lingulate with broad, rounded apex, often fringed at the tip. This species usually forms loose carpets in shaded habitats, especially in coniferous forests. It is also common in rich fens among willows and alders. It prefers moist sites but it is seldom found near water level (McQueen, 1990).

The species studied in the genus *Drepanocladus* include *Drepanocladus fluitans* and *Drepanocladus revolvens*. *Drepanocladus fluitans* is a loose, green, yellow green, or brown shiny plant. The leaves are rather spread apart, 3-4 mm long, oblong-lanceolate. Setae and capsules are 50-75 mm and 2-3 mm long, respectively. It usually grows on wet soils of

ditches, swamps, bogs, meadows, swales, or lake margins, often submerged. It is a nearly cosmopolitan species (Crum, 1973).

Drepanocladus revolvens is characterized by rather robust plants in dense, glossy, typically reddish to blackish-purple tufts. Leaves are usually crowded, smooth when dry, 3-4 mm long, concave, lanceolate, or ovate lanceolate. Setae and capsules are 30-55 mm and 2-3 mm long, respectively. It usually grows on wet soils in swampy places, near springs or margins of lakes. It is common in alkaline bogs (Crum, 1973).

The species studied belonging to the genus *Calliergon* include *Calliergon cordifolium* and *Calliergon stramienum. Calliergon cordifolium* is a rather robust plant in soft, loose, green or yellow-green masses about 7-15 cm high. Stem leaves are not crowded, erect-spreading, 1.8-3 mm long, cordate-ovate. Setae and capsules are 35-65 mm and 2-3 mm long, respectively. It grows on various substrata in or at the margins of temporary pools, along streams, in swamps, swales, or peat bogs. It is often emergent or temporarily submerged (Crum, 1973).

Calliergon stramienum is a relatively slender plant in deep, loose or dense, somewhat shiny, green or more often yellowish to straw-colored masses. Stems are 5-12 cm high (or rarely as much as 30 cm), sparsely branched and erect ascending. Stem leaves are somewhat crowded, 1.2-2 mm long, oblong or oblong-ovate. Setae are 35-45 mm long, red-yellow or red; capsules are about 2 mm long. It grows in shallow pools, or damp depressions in fens, bogs, or boggy woods (Crum, 1973).

The only species studied belonging to the genus *Aulacomnium* is *Aulacomnium palustre.* This is a fairly robust plant forming loose or dense yellow, yellow-green or yellow-brown tufts 3-9 cm high. The leaves are erect-spreading, twisted and contorted when dry, 2-4 mm

long, keeled, oblong-lanceolate, acute or acuminate. Setae are 25-45 mm long; capsules are 2.5-4 mm long, strongly inclined to horizontal and curved. It is very common on various substrata, particularly soil or humus, in swamps, fens, and burned-over bogs (Crum, 1973).

The only terrestrial moss species analyzed is *Callicladium haldanianum*. This moss occurs in loose, irregularly branching, dark to brownish green mats. Leaves are loosely imbricate, concave, ovate- to oblong-lanceolate, narrowed to a short acumination. Capsules are sub-erect or inclined, somewhat curved (Crum, 1973). This species is common on soil, humus, and especially rotting wood in moist shaded places.

Isotopic systematic of moss cellulose

The oxygen isotopic composition of moss cellulose has been investigated only in three studies (Brenninkmeijer et al., 1982; Aravena and Warner, 1992; Menot-Combes et al., 2002). Brenninkmeijer et al. (2002) analyzed the oxygen and hydrogen isotopic composition of cellulose extracted from modern bog plant species in order to interpret the values obtained from a peat core extracted from the eastern part of the Netherlands. Their analyses concerned both vascular plants and mosses. They found remarkably lower δD and $\delta^{18}O$ values in *Sphagnum* mosses than those measured in vascular plants. The highest depletion in D and ^{18}O was found for *Sphagnum cuspidatum*, which grows submerged. *Sphagnum molle*, which favors dry habitat, showed a $\delta^{18}O$ value 2‰ higher than *S. cuspidatum*. These results are consistent with the leaf water enrichment model because evapotranspiration produces higher δ-values in the water available for photosynthesis and it increases as the relative humidity decreases.

Aravena and Warner (1992) analyzed the oxygen isotopic composition of cellulose from five species of *Sphagnum* growing in three peatlands in Ontario. They found that species growing on hummocks (*S. fuscum* and *S. capilliofolium*) had significantly higher $\delta^{18}O$ values than species growing in depressions (*S. fallax*, *S. magellanicum*, and *S. papillosum*), confirming the importance of the bog microtopography in controlling evapotranspiration rates.

The most complete study concerning the effect of climate on the isotopic composition of cellulose from modern bog plants was conducted by Menot-Combes et al. (2002). These authors investigated the isotopic composition of modern plant species and bog surface waters along an altitudinal transect in Switzerland. They found that, for most of the species studied, cellulose $\delta^{18}O$ values decreased with altitude, following the trends in precipitation and in surface water samples. However, some species, including *Sphagnum cuspidatum*, did not reflect changes in climatic parameters associated with altitude. The variability in $\delta^{18}O$ values of *Sphagnum cuspidatum* was found to be dependent upon changes in relative humidity, which was not correlated with altitude. This study underlines the importance of species-specific studies when interpreting $\delta^{18}O$ values of peat deposits as a record of past climate changes.

The number and limitations of these studies do not allow a complete understanding of the factors controlling the variability of $\delta^{18}O$ values in moss cellulose. As mentioned before, these studies are restricted to ombrogenic bogs where meteoric precipitation is the only form of water supply. None of these studies investigated the possibility of the use of groundwater as a source for moss cellulose biosynthesis. By comparing the $\delta^{18}O$ values of moss cellulose, pond water, and groundwater, this research will, therefore, provide a better understanding of

the relationship between water source and cellulose isotopic composition. This investigation is required in order to correctly interpret peat cellulose $\delta^{18}O$ values that will be obtained in future studies for paleoclimate reconstructions of the Great Lakes region.

HYPOTHESIS

The working hypothesis of this study is that the isotopic composition of moss cellulose reflects, to some extent, the isotopic composition of rainwater. This hypothesis derives from the fact that photosynthetic organisms that synthesize cellulose in lakes and ponds utilize oxygen and hydrogen isotopes from surrounding water, thereby recording the isotopic abundance of rainwater. The relationship between the isotopic composition of cellulose and that of the water used for biosynthesis is, however, complex and not completely understood. Consequently, this relationship needs to be assessed. Moreover, the isotopic composition of the water source of mosses must be analysed and characterized to determine the possible effects of evaporation, groundwater inflow, and snowmelt mixing. Hence, the goal of this study is to establish a consistent relationship between the isotopic composition of moss cellulose and that of rainwater. Because peat is mainly composed of decayed mosses, this relationship can be applied for paleoclimate reconstructions of the Lake Superior area from the study of the isotopic composition of peat cellulose.

METHODS

SAMPLING SITES

The selected sites are located in the Michigan's Upper Peninsula along the southern coast of Lake Superior (Figure 6). According to Koppen's classification (Ackerman 1941), which is based upon vegetation types, this region has a climate type called humid continental cool summer. It is characterized by an average temperature of the coldest month of -9°C, by an average temperature of the warmest month of 18°C, and by the absence of a dry season during the year. The selected sites experience, therefore, cold, snowy winters, cool or moderately warm summers, and evenly distributed precipitation. The yearly rainfall amount ranges from 700 to 800 mm with the summer months (May through September) accounting for approximately 50% of the annual precipitation. The range in annual temperature is large and the region is subjected to rapid air masses exchanges and a high frequency of atmospheric disturbances (Eichenlaub, 1979).

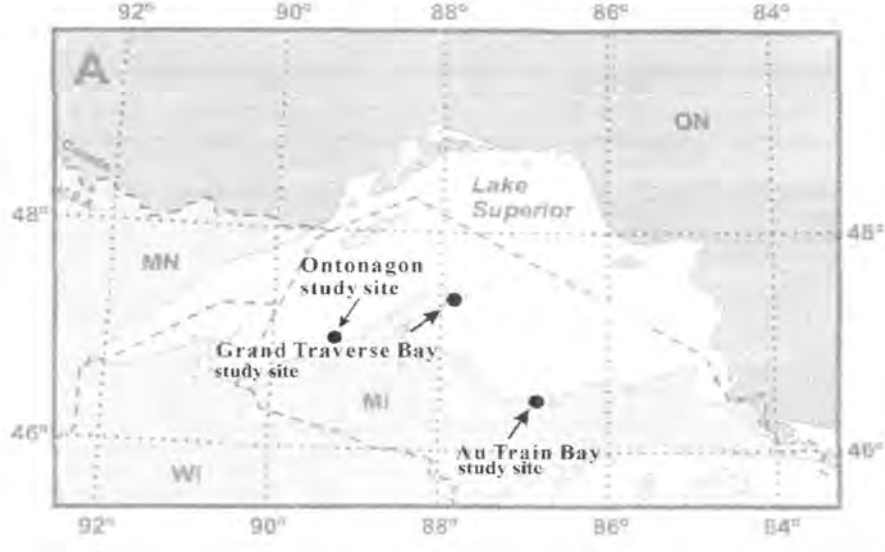

Figure 6. Map of Lake Superior showing the location of the sampling sites (modified from Johnston et al., 2003).

The first sampling site (47°08'59'' N and 88°14'40'' W) is a swale on the Grand Traverse Bay embayment, a 4 km long and 2.4 km wide strandplain, on the north-east side of the Keweenaw Peninsula (Figure 7). According to the classification of Moore and Bellamy (1974), this peatland can be classified as a minerotrophic fen on the basis of its pH of about 5.4. Therefore, its water balance is considerably influenced by groundwater. Prior to European settlement, both white pine (*Pinus strobus*) and red pine (*Pinus resinosa*) were found on the beach ridges of this site. Today, jack pine (*Pinus banksiana*) is the dominant species on the beach ridges along the shoreline. Scattered black spruce (*Picea mariana*) and, occasionally, tamarack (*Larix laricina*) are dominant in the swales. Other species common in swales include sweet bay (*Myrica gale*), leatherleaf (*Chamaedaphne calyculata*), bog birch

Figure 7. Grand Traverse sampling site.

(*Betula pumila*), speckled alder (*Alnus rugosa*), bog rosemary (*Andromedea glaucophylla*), bog laurel (*Kalmia polifolia*), labrador tea (*Ledum groenlandicum*), willow (*Salix pedicellaris*), running bog sedge (*Carex oligosperma*), blue joint grass (*Calamagrostis canadensis*), blue flag (*Iris versicolor*), pitcher plant (*Sarracenia purpurea*), and a few species of sphagnum mosses (*Sphagnum spp.*).

The second sampling site is a swale (46°25'43'' N and 86°51'28'' W) located on the Au Train Bay embayment, which is a 5 km long, 2.5 km wide strand plain (Figure 8). This peatland, having a pH of about 4.8, can be classified as a transitional fen. This kind of wetland is intermediate between mineral-nourished (minerotrophic) and precipitation-dominated (ombrotrophic) (Moore and Bellamy, 1974). The beach ridges of this complex are generally low to moderate in height (0.5-1.5 m). White pine, red pine, and jack pine

Figure 8. Au Train sampling site.

dominated the ridges whereas northern white cedar (*Thuja occidentalis*) and spruce (*Picea spp.*) dominated the swales prior to European settlement. Today, red pine, jack pine, white pine, red oak (*Quercus rubra*), and white spruce (*Picea glauca*) dominate the ridges, whereas black spruce (*Picea mariana*) and a number of shrubs dominate many of the swales. With the exception of mountain holly (*Nemopanthus mucronata*), large cranberry (*Vaccinium macrocarpon*), and flat-leaved bladderwort (*Utricularia intermedia*), the other species common in the swales are the same found in Grand Traverse Bay.

The third selected swale is located 4 miles northeast of Ontonagon (46°53'57'' N and 89°14'49'' W), on the south-west side of the Keweenaw Peninsula (Figure 9). This peatland has a pH of about 5.7 and therefore can be classified as another minerotrophic fen

Figure 9. Ontonagon sampling site.

(Moore and Bellamy, 1974). The beach ridges in this complex are moderate in height, ranging from 0.5 to 2 m, and are dominated by white Pine, red pine, eastern hemlock (*Tsuga canadensis*), red maple (*Acer rubrum*), paper birch (B*etula papyrifera*), white spruce, and big tooth aspen (*Abies balsamea*). The swales are inhabited by leatherleaf, speckled alder, Michigan holly (*Ilex verticillata*), bog rosemary, and sphagnum mosses. Other common species include large cranberry, running bog sedge, round-leaved sundrew (*Drosera rotundifolia*), pond sedge (*Dulichium arundinaceum*), beak rush (*Rhynchospora fusca*), rattlesnake grass (*Glyceria arundinaceum*), and Northern manna grass (*Glyceria borealis*).

These sites were selected because they represent the same sequences of swales and beach ridges that have been used to reconstruct water levels in Lake Superior (Johnston et al., 2003) and because they are relatively undisturbed by human activities.

Samples of mosses, pond water, groundwater, and moss water (i.e., water obtained by squeezing the mosses in the field) were collected at each site in May, July, October 2003 and monthly from May to August 2004. Samples of mosses were manually picked out from the swales. Water samples were collected with a plastic syringe, filtered in the field with Cameo 0.45 μm nylon filters to prevent the inclusion of organic matter that can interfere with the isotopic analyses, and placed in 20 ml vial. To prevent evaporation, vials were completely filled with water, capped, and sealed with a layer of Parafilm®.

ISOTOPIC ANALYSES

Water samples

The isotopic analyses of water samples were performed on a Thermo Finnigan Delta-Plus XL mass spectrometer installed at the Department of Geological and Atmospheric Sciences at Iowa State University. Values of δD for the water samples were obtained using the zinc reduction method (Coleman et al., 1982). Briefly, glass tubes of 6 mm of outer diameter were loaded with 100 mg of Indiana Zinc (Schimmelmann and DeNiro, 1993) and connected to a vacuum line with an Ultra-Torr® fitting. The ampoules containing zinc were heated for 8 minutes at 250°C to ensure the release of absorbed atmospheric moisture. A small amount of water (2μl) was injected into the system with a syringe by piercing a septum in the glass tube. During the injection of water, the zinc-containing part of the ampoule was immersed in liquid nitrogen to capture the injected water cryogenically at the bottom of the tube. Once water was cryogenically transferred to the bottom of the ampoule, the tube was flame-sealed with a glassblowing torch under vacuum. Prior to performing the isotopic determinations, the tubes were heated at 500°C for 30 minutes to allow the quantitative generation of hydrogen gas from the reaction of water with zinc. The ampoules were scored with a glass knife and the generated hydrogen was then injected to the mass spectrometer via a dual inlet. Each sample was measured in duplicate and two standards were analysed every 10 samples and used to correct for drift. The analytical uncertainty for δD was typically better than 1‰.

Values of $\delta^{18}O$ were determined by equilibrating water samples with CO_2 of known isotopic composition for 24 hours (Epstein and Mayeda, 1953). Flat-bottomed vials were flushed with 0.3% CO_2 in helium for 2 minutes and sealed with a cap containing a septum. 0.5 ml of water was then loaded with the aid of a syringe through the septum. Vials were

placed on a metal block at a constant temperature of 25°C, and they were allowed to equilibrate for 24 hours. The equilibrated CO_2 was analysed by the mass spectrometer on continuous flow mode, using a Gas Bench-II fitted to a Finnigan Delta Plus XL mass spectrometer. Standards were analysed every 12 samples and used to correct for drift. The analytical uncertainty for $\delta^{18}O$ was typically better than 0.1‰. The $\delta^{18}O$ and δD results are expressed relative to V-SMOW.

Cellulose

After being collected, mosses were washed with pure deionised water, freeze-dried for 48 hours, and ground with a mortar and pestle. Subsequently, cellulose was extracted from the pulverized samples following the techniques developed by Green (1963) and Wolfe et al. (2001). The extracted cellulose (referred to as α-cellulose or alkali-resistant cellulose) was the substrate used for isotopic determinations. Duplicates were extracted every 10 samples.

The first step in the extraction is the removal of lipids. Pulverized mosses (0.25 g) were placed in a beaker containing 200 ml of 2:1 benzene:methanol mixture. After 24 hours, the samples were filtered and treated with 200 ml of acetone for another 24 hours. The remaining liquid was then eliminated and the samples were left in a fume hood to dry. The removal of lignin was the subsequent step and was accomplished by treating the samples with 0.75-1.0 ml of acetic acid and 0.75-1 g of sodium chlorite at 70°C. The treatment was repeated on an hourly basis until the samples were pale grey to yellowish-grey or white (usually 5 times). The samples were then rinsed with distilled water 5-10 times. The last step, the removal of hemicellulose, was achieved by placing the samples in 200 ml beakers containing a 17% solution of sodium hydroxide and distilled water for 45 minutes. Samples were then rinsed

with deionised water and immersed in 200 ml beakers containing a 10% solution of acetic acid and distilled water for 15 minutes. Washed samples were finally put in a plastic thimble and freeze-dried for 48 hours.

Since cellulose strongly absorbs water, a proper drying of the samples is important for $\delta^{18}O$ analysis. Therefore, small aliquots (10-15 mg) of α-cellulose were placed in silver-foil capsules, dried at 50°C overnight, and loaded onto a Finnigan TC/EA instrument. The oxygen isotopic composition was determined by an on-line continuous-flow mode. Cellulose was quantitatively converted into carbon monoxide via pyrolysis at 1200°C, which was facilitated by the presence of glassy carbon in the Finnigan TC/EA instrument. The oxygen isotopic composition was measured on the same mass spectrometer used for the analysis of the water samples. Each cellulose sample was measured in duplicate and a set of three standards (benzoic acids and IAEA cellulose) were measured every six-ten samples. The analytical uncertainty for cellulose $\delta^{18}O$ values was typically better than 0.6‰.

RESULTS

WATER SAMPLES

The $\delta^{18}O$ and δD values of the water samples collected in Au Train, Grand Traverse, and Ontonagon are reported in Tables 1, 2, and 3. Figures 10-30 include plots of monthly $\delta^{18}O$ and δD values for pond water, moss water, groundwater, and for the monthly weighted average $\delta^{18}O$ and δD values of rainwater. These rainwater data were obtained from the online "isotopes in precipitation calculator" (**http://es.ucsc.edu/%7egbowen/form.html**). In all three sampling localities, the $\delta^{18}O$ and δD values for moss water are not statistically different from those for pond water (P values>0.5, t-unpaired tests, n=7).

The pond water samples collected in Au Train show δD values ranging from a low value of –90.5‰ in May 2004 to a high value of –59.7‰ in August 2004. The average δD value is –72.8‰ (Figure 10). The $\delta^{18}O$ values range from –11.14‰ (May 2004) to –6.76‰ (July 2003) and show an average value of –8.84‰ (Figure 11). A comparison between rainwater data and pond water data shows similar seasonal trends (Figures 10, 11), although pond water δ-values are systematically lower. The offset between the two curves is large early in the growing season (≈27‰ for δD and 2.4‰ for $\delta^{18}O$ in May 2003) and tends to decrease as the season proceeds (Figures 10, 11). The δD values for the moss water samples range from -101.7‰ (October 2003) to –56.9‰ (August 2004), showing a higher variability than the pond water samples and an average value of –74.3‰ (Figure 12). The $\delta^{18}O$ values for the same samples range from –13‰ (October 2003) to –6.82‰ (August 2004) and show an average value of –9.23‰ (Figure13). The samples of groundwater from this site show a much smaller range of values, varying by only 4.1‰ for δD and 2.14‰ for $\delta^{18}O$

Table 1. δD and $\delta^{18}O$ values (‰) for water samples collected in Au Train.

Au Train	*Pond water*		*Moss water*		*Groundwater*	
	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$
May 2003	-83.1	-10.26	-82.6	-10.15	-89.8	-13.49
July 2003	-61.1	-6.76	-62.8	-7.43	-89.5	-11.35
October 2003	-85.3	-10.58	-101.7	-13.00	-87.0	-11.43
May 2004	-90.5	-11.14	-85.8	-11.31	-89.8	-11.84
June 2004	-68.2	-8.68	-71.5	-8.62	-88.2	-11.97
July 2004	-62.0	-7.48	-58.7	-7.28	-85.7	-12.42
August 2004	-59.7	-7.01	-56.9	-6.82	-86.9	-12.37

Table 2. δD and $\delta^{18}O$ values (‰) for water samples collected in Grand Traverse. July 2004 moss water values correspond to the *Drepanocladus fluitans* sample.

Grand Traverse	*Pond water*		*Moss water*		*Groundwater*	
	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$
May 2003	-96.8	-14.19	-94.4	-15.25	n.a.	n.a.
July 2003	-92.7	-11.32	-87.4	-10.50	n.a.	n.a.
October 2003	-94.4	-11.64	-94.4	-12.34	-91.1	-12.04
May 2004	-98.9	-12.80	-97.0	-12.99	-90.3	-11.66
June 2004	-83.6	-11.18	-80.4	-11.23	-94.0	-12.44
July 2004	-84.0	-11.97	-78.5	-11.76	-91.5	-13.33
August 2004	-79.9	-11.42	-78.6	-11.81	-91.7	-13.34

Table 3. δD and $\delta^{18}O$ values (‰) for water samples collected in Ontonagon. May and August 2004 moss water values correspond to the *Calliergon cordifolium* samples.

Ontonagon	*Pond water*		*Moss water*		*Groundwater*	
	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$
May 2003	-94.7	-13.87	-94.6	-15.27	n.a.	n.a.
July 2003	-75.7	-8.97	-71.2	-9.51	n.a.	n.a.
October 2003	-83.6	-10.39	-83.0	-10.55	-88.4	-11.17
May 2004	-95.1	-12.21	-93.4	-12.10	-97.4	-12.70
June 2004	-79.4	-10.42	-74.4	-10.45	-100.6	-12.96
July 2004	-78.4	-11.02	n.a.	n.a.	-89.2	-12.51
August 2004	-80.5	-11.32	-79.7	-11.14	-89.7	-12.53

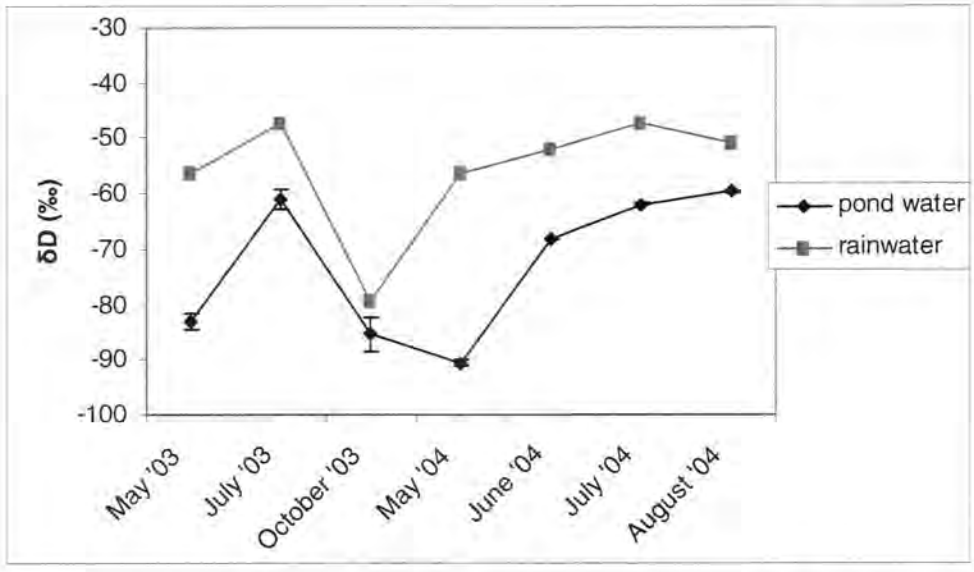

Figure 10. δD values of pond water samples collected in Au Train.

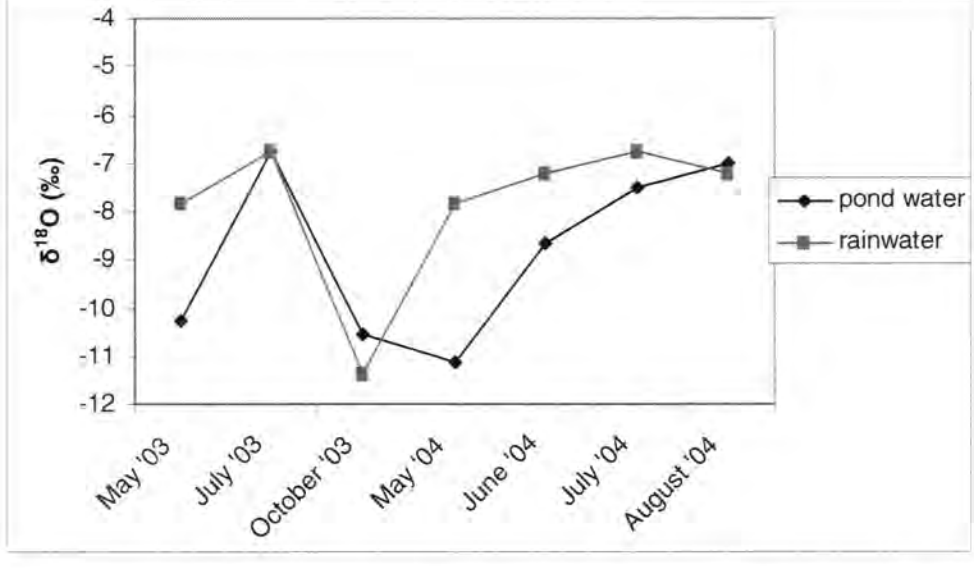

Figure 11. $\delta^{18}O$ values of pond water samples collected in Au Train.

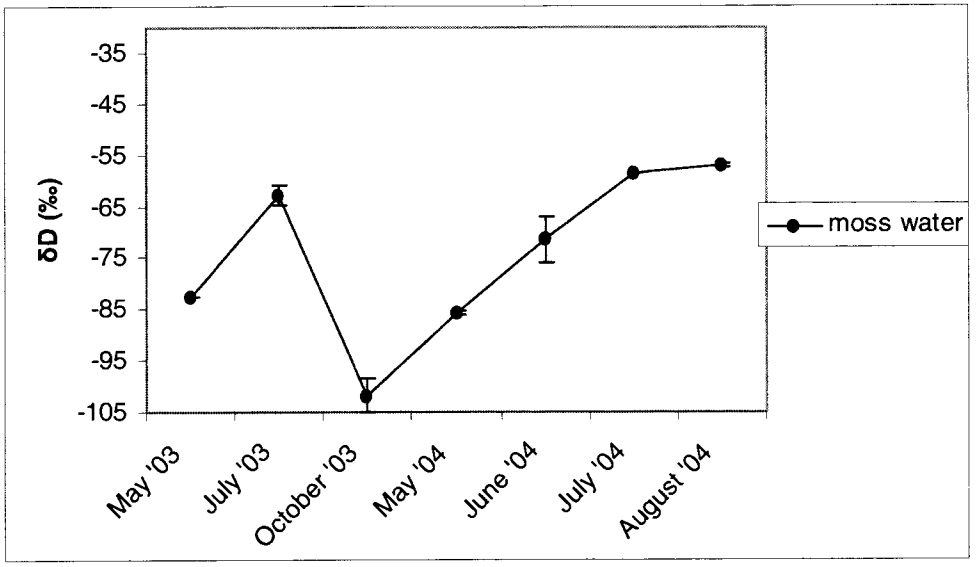

Figure 12. δD values of moss water samples collected in Au Train.

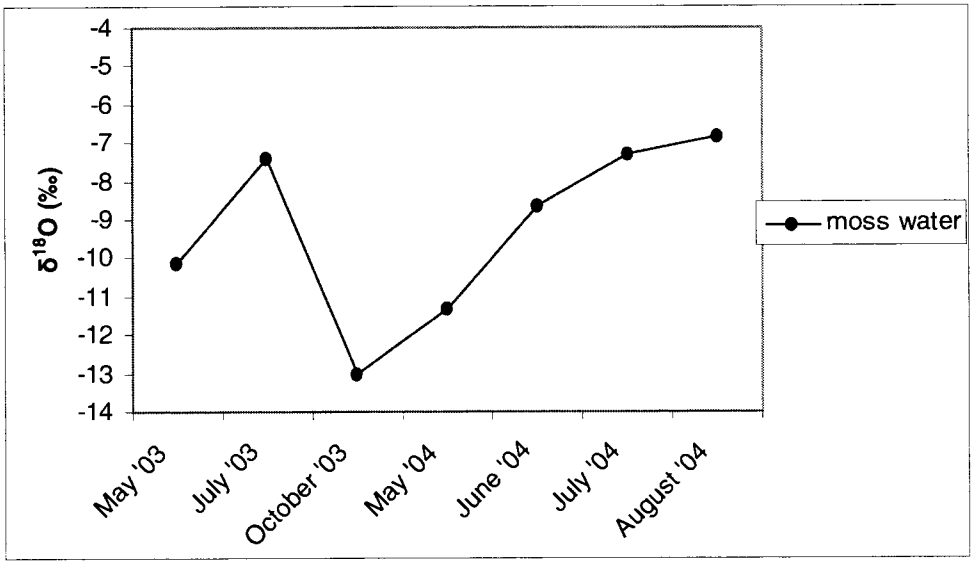

Figure 13. $\delta^{18}O$ values of moss water samples collected in Au Train.

and showing an average value of –88.1‰ and –12.12‰ for δD and $\delta^{18}O$, respectively (Figures 14, 15). The $\delta^{18}O$-δD graph of the water data from this site shows that pond water plots along a line characterized by a slope similar to that of the LMWL (R^2=0.98), whereas the groundwater δ-values cluster around the LMWL and show a small variability in $\delta^{18}O$ and δD (Figure 16). All water samples plot within the uncertainty associated with the LMWL calculated for Mead, Nebraska by Harvey (2001) (Figure 16).

The pond water and moss water samples collected in Grand Traverse show average δD and $\delta^{18}O$ values considerably lower than the average values for their Au Train counterparts. In addition to that, the δ-values of the groundwater samples are not statistically different from the δ-values of the pond water samples (P value=0.12, t-unpaired tests, n=5). Whereas pond water δD values range from –98.9‰ (May 2003) to –79.9‰ (August 2004) (Figure 17), the $\delta^{18}O$ values range from –14.19‰ (May 2003) to –11.18‰ (June 2004) (Figure 18). Rainwater and pond water isotope data show a remarkable offset during most of the growing season, exhibiting systematically lower pond water δ-values. The only exception is the isotopic values of October samples, which are almost identical (Figures 17, 18). The samples of moss water show a similar trend with the lowest δD value (-97‰) shown by the sample collected in May 2004 and the highest δD value (-78.5‰) shown by the sample collected in July 2004 (Figure 19). Moss water $\delta^{18}O$ values range from –15.25‰ (May 2003) to –10.5 ‰ (July 2003) (Figure 20). The groundwater isotope data (available only for the months October 2003, May, June, July, and August 2004), show an average δD value of –91.7‰ and an average $\delta^{18}O$ value of –12.56‰. The range of these data is very small (2.9‰ for the δD values and 1.68‰ for the $\delta^{18}O$ values) (Figures 21, 22). The $\delta^{18}O$-δD graph shows that pond water samples lie close to the LMWL and close to the groundwater δ-values (Figure 23).

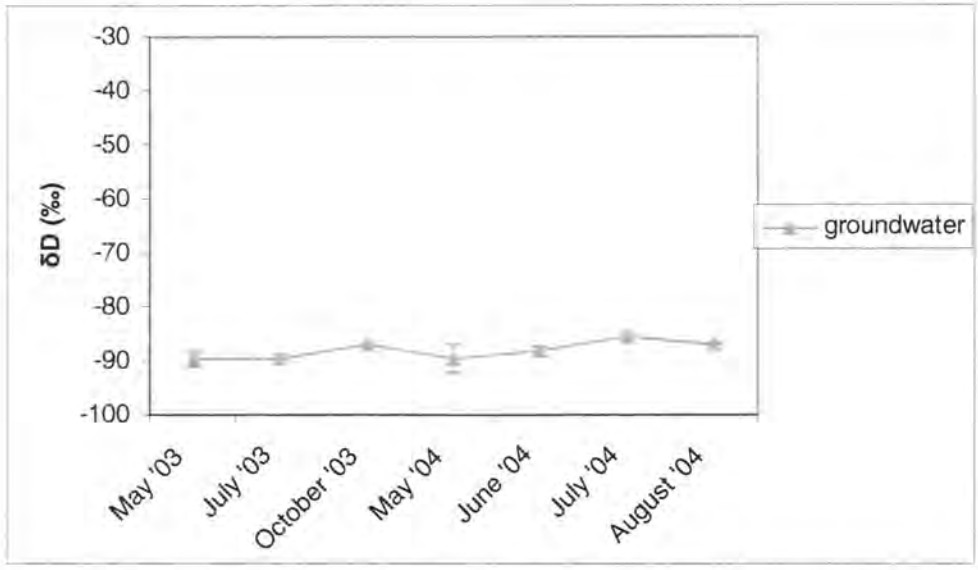

Figure 14. δD values of groundwater samples collected in Au Train.

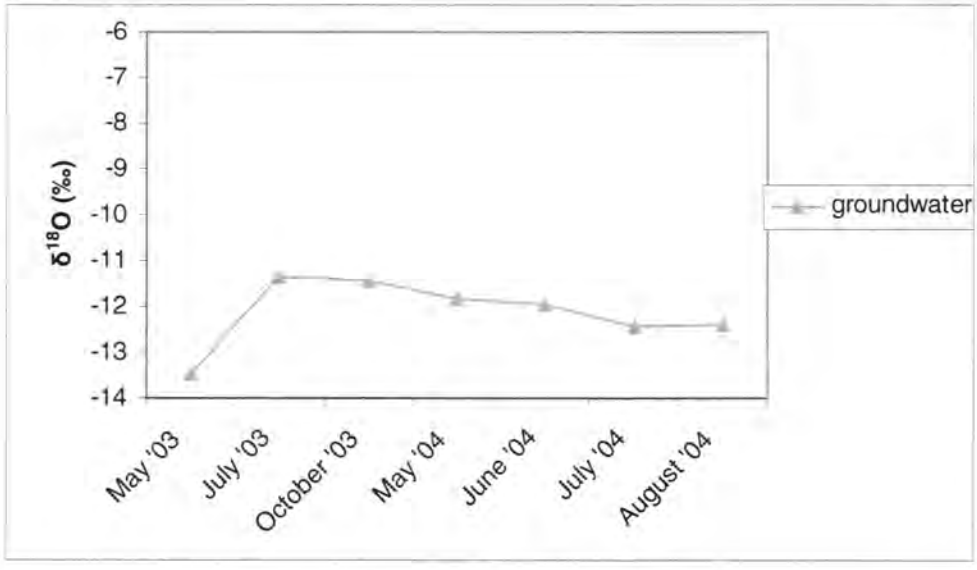

Figure 15. $\delta^{18}O$ values of groundwater samples collected in Au Train.

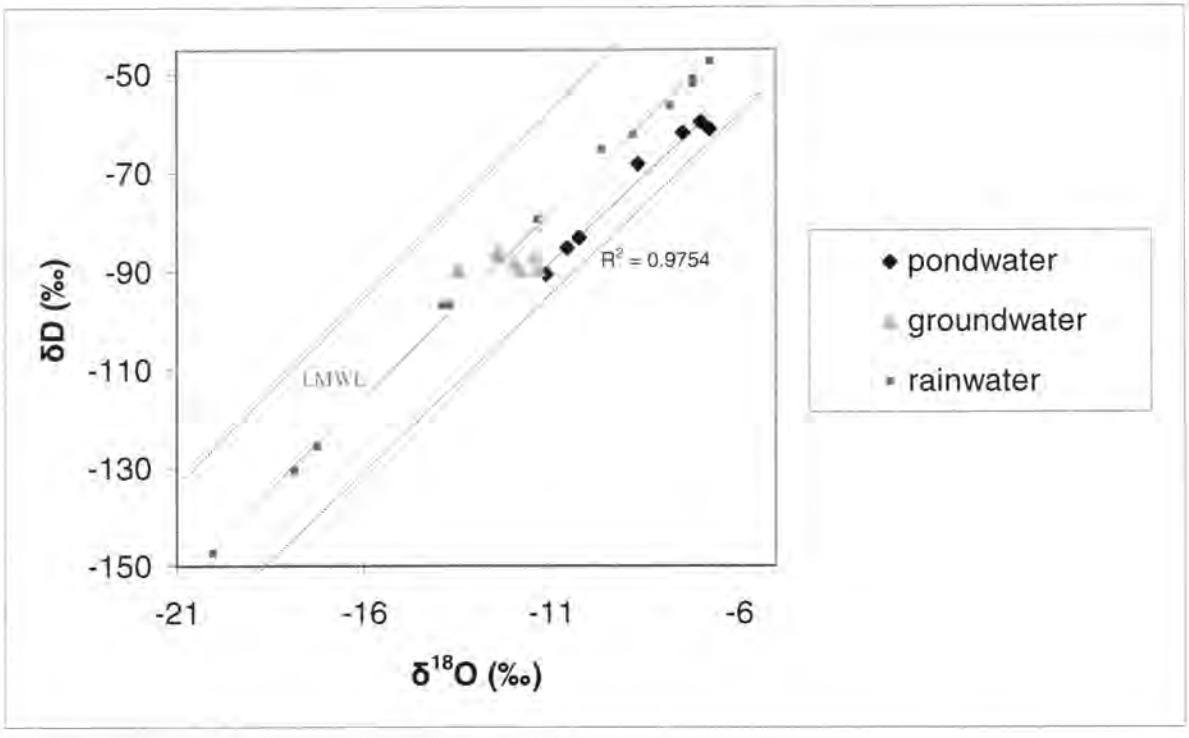

Figure 16. δD-$\delta^{18}O$ plot of water samples collected in Au Train. The dashed lines represent the uncertainty associated with the MWL calculated by Harvey (2001).

The pond water and moss water samples collected in Ontonagon show average $\delta^{18}O$ and δD values greater than the average values reported for Grand Traverse samples, but smaller than those for the Au Train samples. In Ontonagon, whereas the δD values of pond water samples range from –95.1‰ (May 2004) to –75.7‰ (July 2003) (Figure 24), the $\delta^{18}O$ values range from –13.87‰ (May 2003) to –8.97‰ (July 2003) (Figure 25). The average pond water δ-values are –83.9‰ for δD and –11.17‰ for $\delta^{18}O$. Rainwater and pond water isotope data show a seasonal trend and an offset similar to those observed in Grand Traverse samples (Figures 24, 25). The average values for the moss water are similar to those of pond water, but the variability in isotopic values is higher for moss water. This higher variability is due to the high δ-values recorded for the moss water in July 2003 (Fig. 26, 27). Average

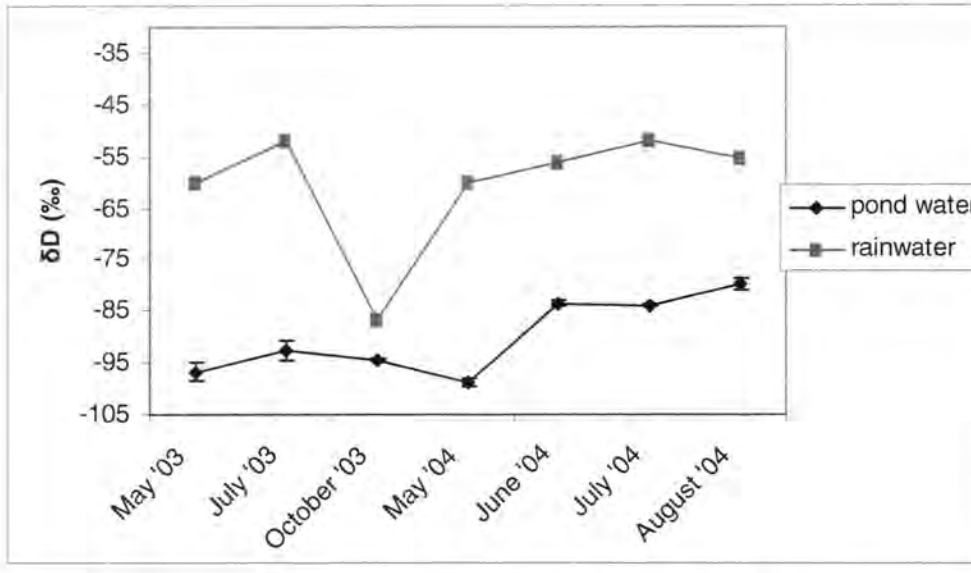

Figure 17. δD values of pond water samples collected in Grand Traverse.

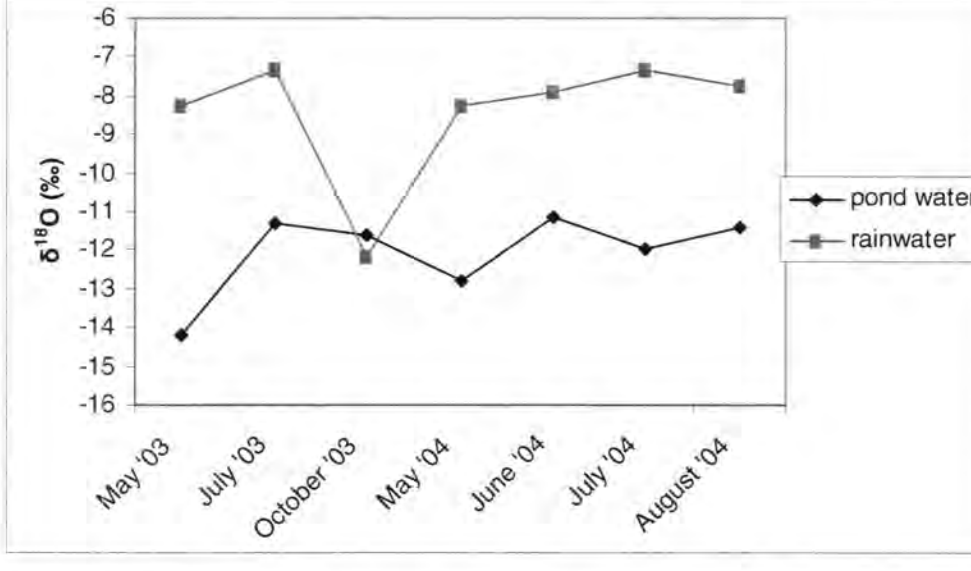

Figure 18. $\delta^{18}O$ values of pond water samples collected in Grand Traverse.

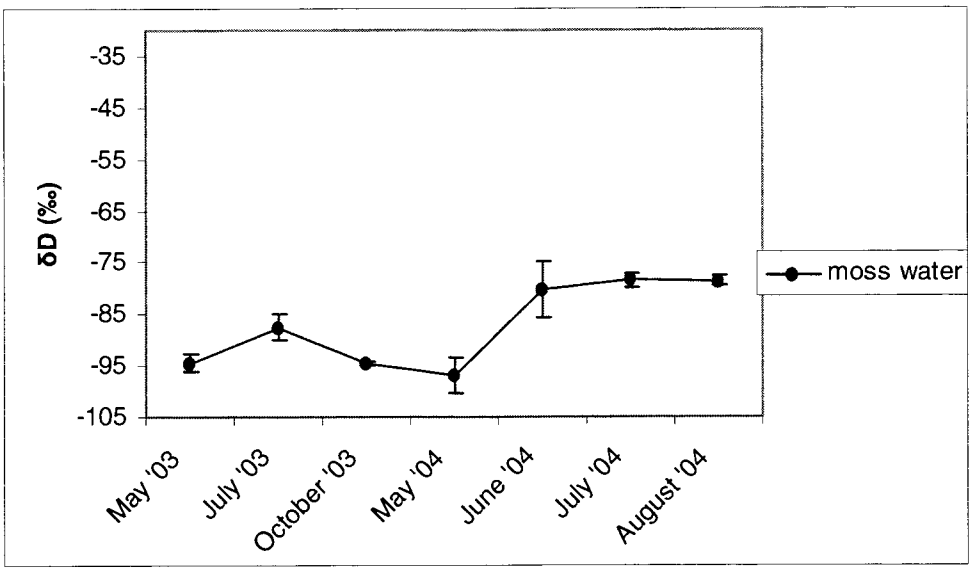

Figure 19. δD values of moss water samples collected in Grand Traverse.

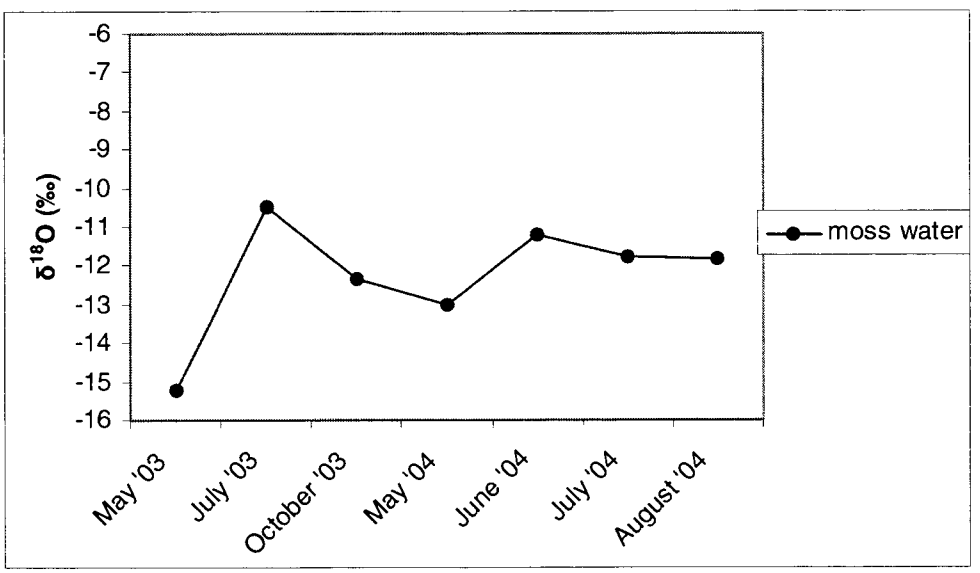

Figure 20. $\delta^{18}O$ values of moss water samples collected in Grand Traverse.

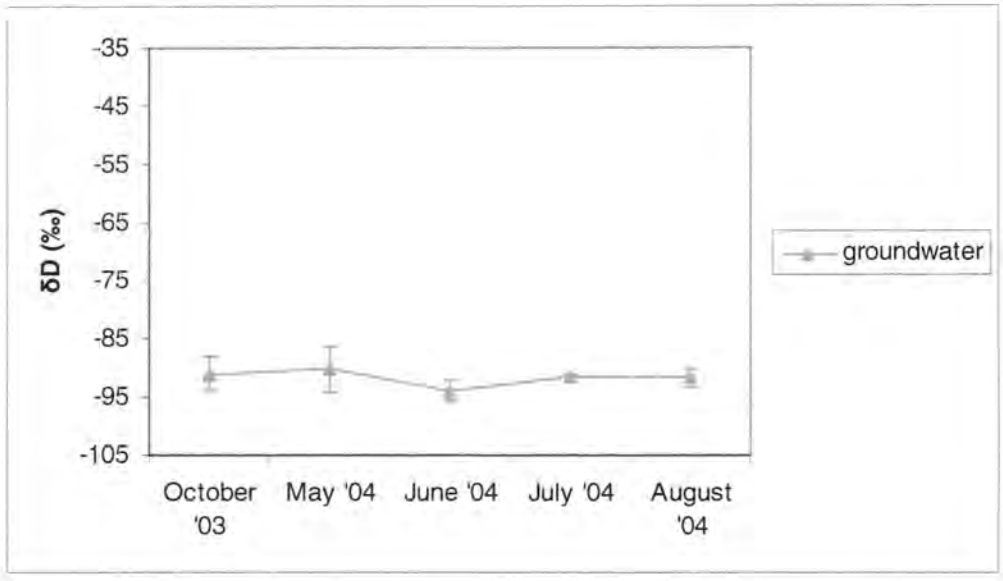

Figure 21. δD values of groundwater samples collected in Grand Traverse.

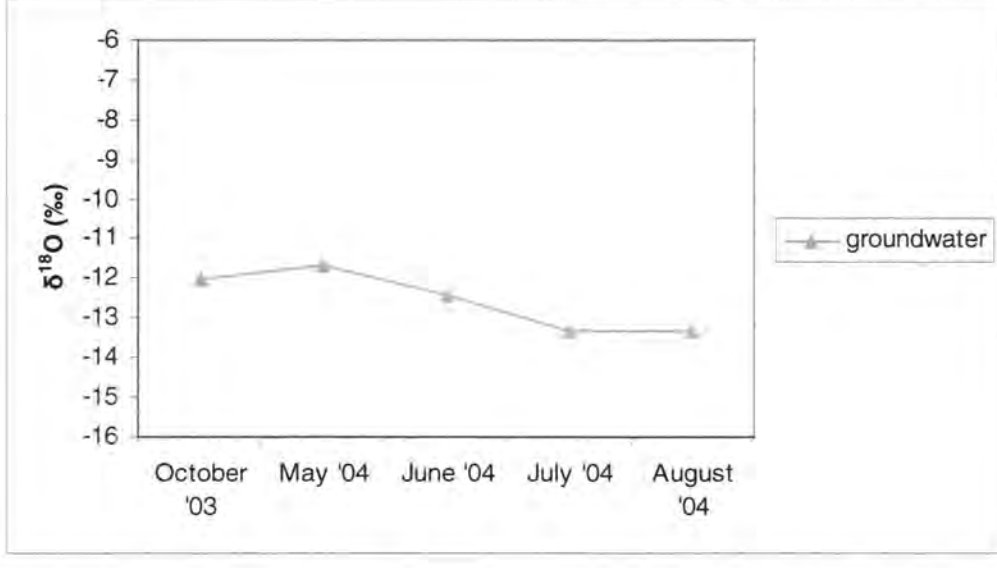

Figure 22. $\delta^{18}O$ values of groundwater samples collected in Grand Traverse.

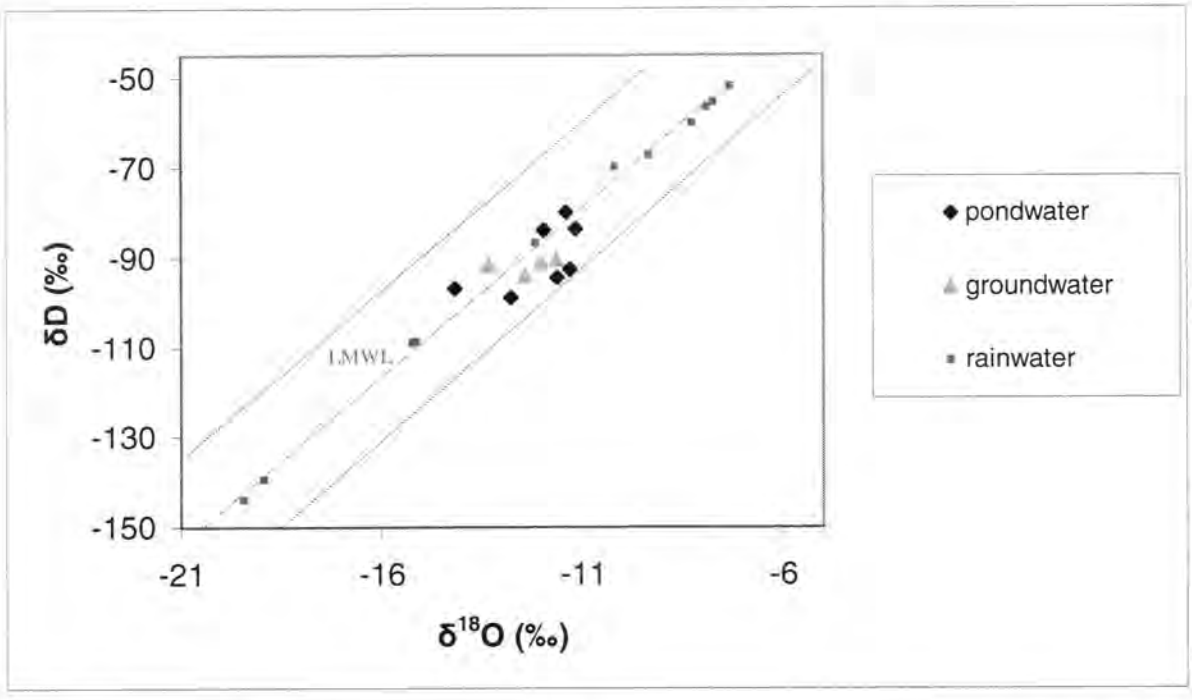

Figure 23. δ D-δ^{18}O plot of water samples collected in Grand Traverse. The dashed lines represent the uncertainty associated with the MWL calculated by Harvey (2001).

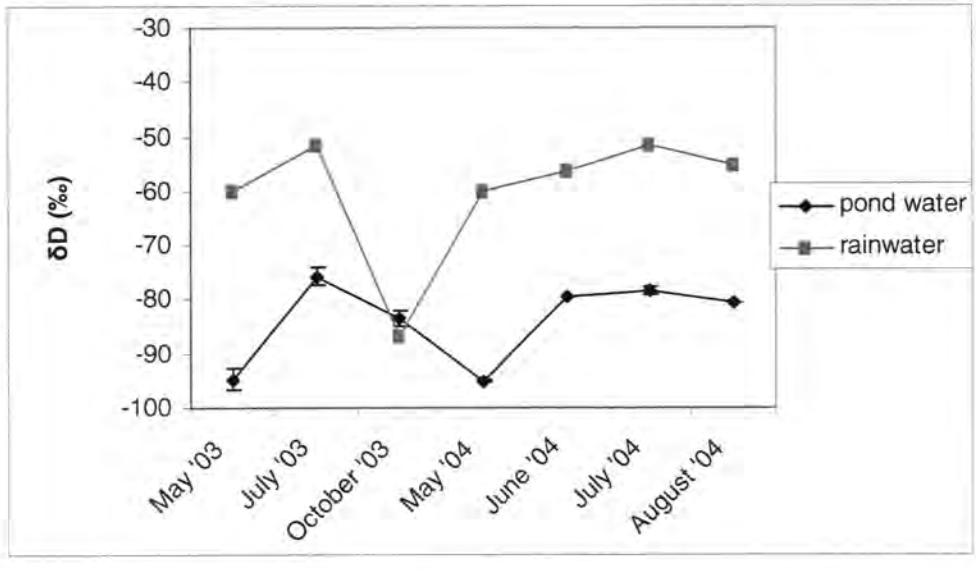

Figure 24. δD values of pond water samples collected in Ontonagon.

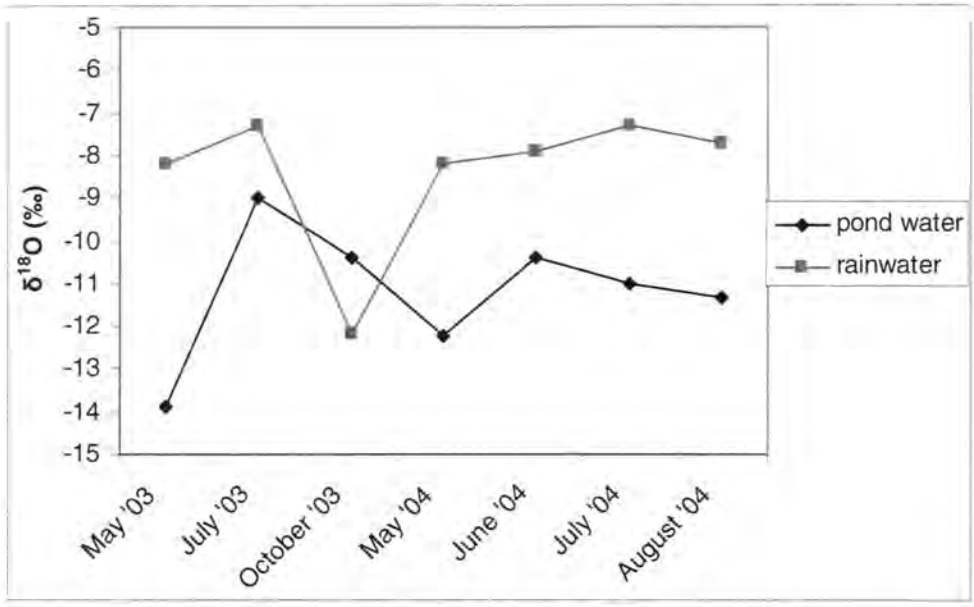

Figure 25. $\delta^{18}O$ values of pond water samples collected in Ontonagon.

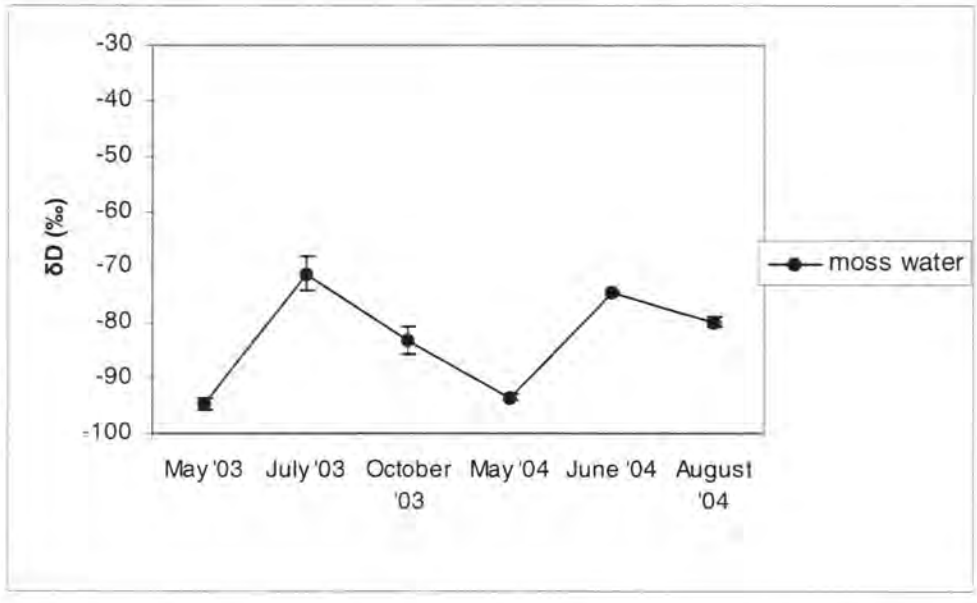

Figure 26. δD values of moss water samples collected in Ontonagon.

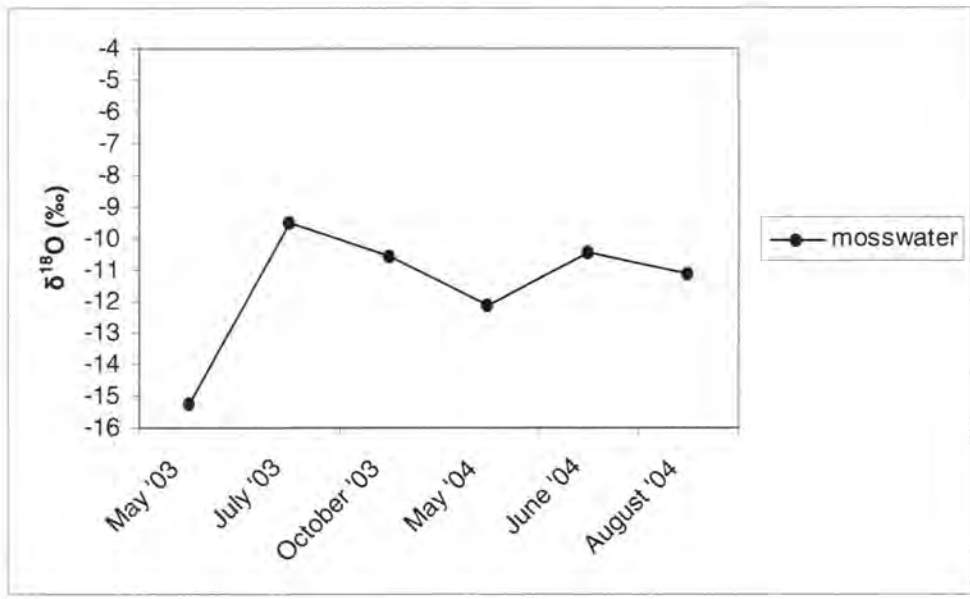

Figure 27. $\delta^{18}O$ values of moss water samples collected in Ontonagon.

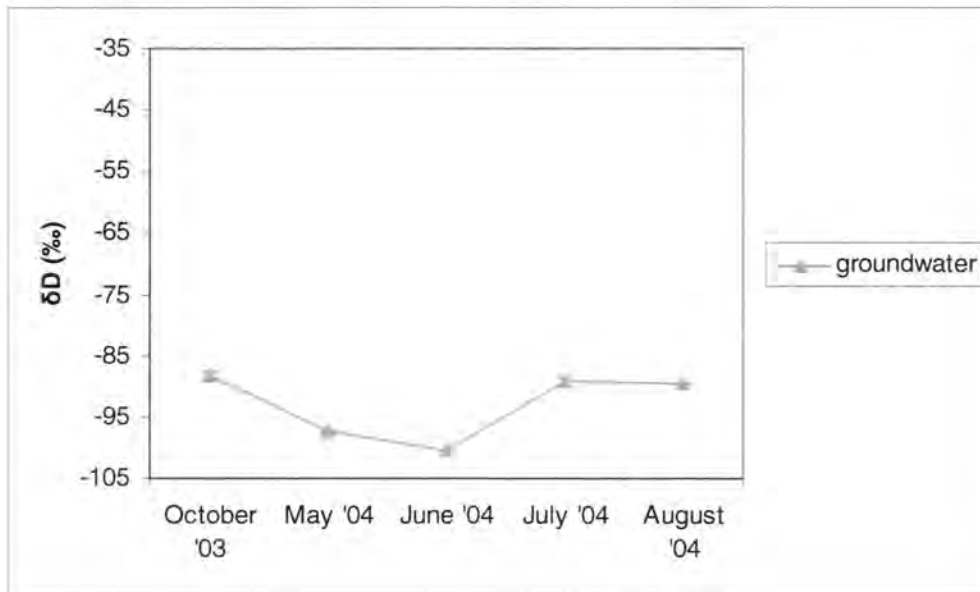

Figure 28. δD values of groundwater samples collected in Ontonagon.

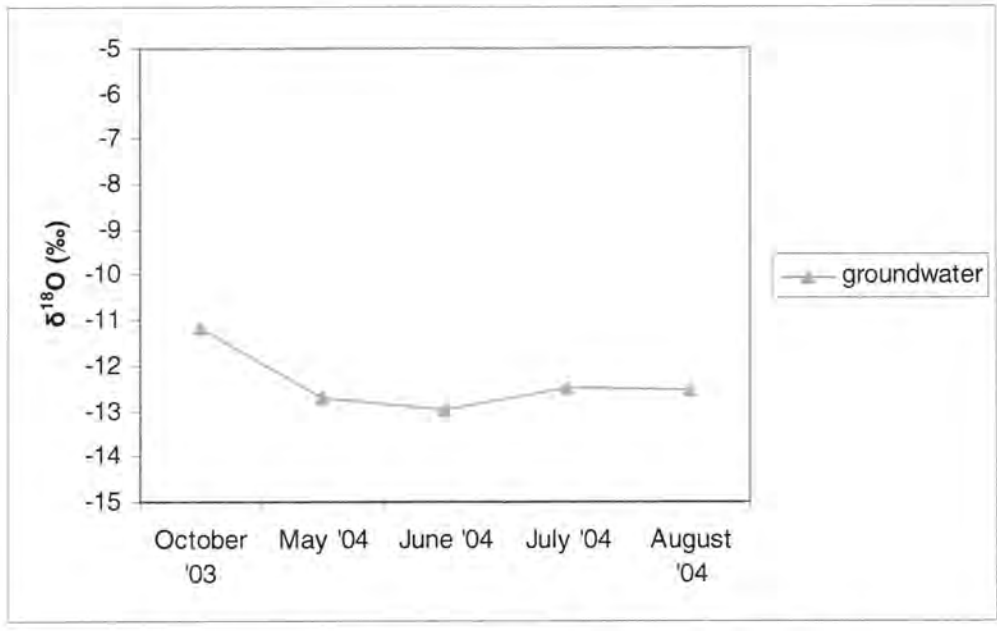

Figure 29. $\delta^{18}O$ values of groundwater samples collected in Ontonagon.

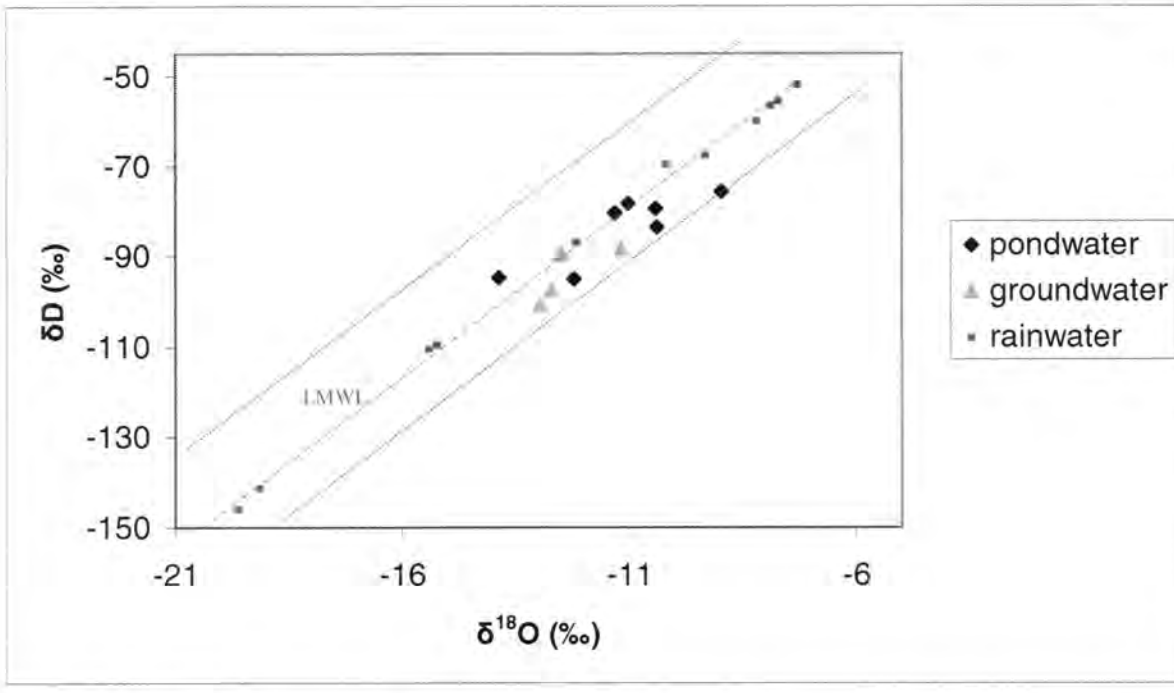

Figure 30. δ D-$\delta^{18}O$ plot of water samples collected in Ontonagon. The dashed lines represent the uncertainty associated with the MWL calculated by Harvey (2001).

groundwater δ-values (δD= –93.1‰; $\delta^{18}O$= –12.37‰) are similar to those recorded in Grand Traverse (Figures 28, 29). Similar to the other two sampling localities, the $\delta^{18}O$-δD plot shows that pond water and groundwater samples plot within the uncertainty associated with the LMWL of Harvey (2001) (Figure 30).

MOSS CELLULOSE

The $\delta^{18}O$ values of cellulose extracted from the different moss species collected each month in the three sites are reported in Tables 4, 5, and 6. Figures 31 and 33 show the $\delta^{18}O$ values for cellulose and pond water obtained in Au Train and Grand Traverse for each sampled month. Figure 32 is a plot of the $\delta^{18}O$ values of moss cellulose against the $\delta^{18}O$ values of pond water for the Au Train samples.

Whereas $\delta^{18}O$ values obtained from the samples collected in 2004 are the result of a single analysis, the values of the samples collected in 2003 represent the averages of two different analyses and different duplicates obtained during the cellulose extractions. In addition, the values shown for the mosses collected in May 2003 represent the average of the values obtained for two different mosses of the same species. Because of this, the analytical uncertainty for the samples collected in 2003 is higher than that of the samples collected in 2004.

The mosses collected in Au Train belong to the same species (*Sphagnum cuspidatum*) except for the sample collected in July 2003, which corresponds to *Sphagnum centrale*. The $\delta^{18}O$ values for the moss cellulose of Au Train range from a low value of 17.2‰ in August 2004 to a high value of 19.42‰ in May 2003. The range of variability, therefore, is 2.2‰. The average $\delta^{18}O$ value for the mosses at this locality is 18.27‰.

Table 4. $\delta^{18}O$ values (‰) for mosses collected in Au Train. It is also reported the enrichment factor (‰) of moss cellulose with respect to the average $\delta^{18}O$ value of pond water.

Au Train	*Moss species*	$\delta^{18}O_{cellulose}$	$^{18}\varepsilon_{cell\text{-}average\ pondw.}$
May 2003	*Spagnum cuspidatum*	19.42	28.27
July 2003	*Spagnum centrale*	18.03	26.88
October 2003	*Spagnum cuspidatum*	19.02	27.87
May 2004	*Spagnum cuspidatum*	17.91	26.75
June 2004	*Spagnum cuspidatum*	18.05	26.90
July 2004	*Spagnum cuspidatum*	18.26	27.10
August 2004	*Spagnum cuspidatum*	17.20	26.04

Table 5. $\delta^{18}O$ values (‰) for mosses collected in Grand Traverse. It is also reported the enrichment factor (‰) of moss cellulose with respect to the average $\delta^{18}O$ value of pond water.

Grand Traverse	*Moss species*	$\delta^{18}O_{cellulose}$	$^{18}\varepsilon_{cell\text{-}average\ pondw.}$
May 2003	*Drepanocladus fluitans*	20.31	32.38
July 2003	*Drepanocladus fluitans*	19.15	31.23
October 2003	*Drepanocladus fluitans*	18.41	30.48
May 2004	*Spagnum cuspidatum*	16.92	28.99
June 2004	*Spagnum cuspidatum*	16.43	28.51
July 2004	*Drepanocladus fluitans*	20.23	32.51
	Aulacomnium palustre	20.44	32.30
August 2004	*Spagnum cuspidatum*	15.85	27.93

Table 6. $\delta^{18}O$ values (‰) for mosses collected in Ontonagon. It is also reported the enrichment factor (‰) of moss cellulose with respect to the average $\delta^{18}O$ value of pond water.

Ontonagon	*Moss species*	$\delta^{18}O_{cellulose}$	$^{18}\varepsilon_{cell\text{-}average\ pondw.}$
May 2003	*Calliergon cordifolium*	18.75	29.92
July 2003	*Drepanocladus revolvens*	22.36	33.53
October 2003	*Sphagnum squarrosum*	17.98	29.15
May 2004	*Calliergon cordifolium*	18.65	29.82
	Callicladium haldanianum	20.63	31.80
June 2004	*Calliergon stramienum*	18.15	29.32
August 2004	*Calliergon cordifolium*	18.99	30.17
	Sphagnum squarrosum	19.98	31.15

The mosses collected in Grand Traverse belong to three different species. Samples of *Drepanocladus fluitans* were collected in May, July, and October 2003. In addition to this species, *Aulacomnium palustre* was collected in July 2004. The samples collected in May, June, and August 2004 correspond to *Sphagnum cuspidatum*. The $\delta^{18}O$ values of cellulose for this site range from a low value of 15.85‰ for the *Sphagnum cuspidatum* collected in August 2004 to a high value of 20.44‰ for the *Aulacomnium palustre* sample collected in July 2004. The average $\delta^{18}O$ value of the three *Sphagnum* samples collected in this locality is 16.4‰ and is 3.22‰ lower than the average $\delta^{18}O$ value obtained for the samples of the other two moss species (19.62‰). This difference is statistically significant (P value=0.002, t-unpaired tests).

The mosses collected in Ontonagon belong to five different species. Samples of May 2003, May 2004, and August 2004 correspond to *Calliergon cordifilium*. Along with this species, a sample of the terrestrial *Callicladium haldanianum* was collected and analyzed in May 2004. The sample of July 2003 corresponds to a *Drepanocladus revolvens*, whereas the

plant collected in October 2003 is a *Sphagnum squarrosum*. A sample of this plant was also collected, along with *Calliergon cordifolium*, in August 2004. The sample collected in June 2004 is a *Calliergon stramienum*. For this site, the $\delta^{18}O$ values of moss cellulose range from a low value of 17.98‰ reported by the *Sphagnum squarrosum* sample collected in October 2003 to a high value of 22.36‰ reported by the *Drepanocladus revolvens* sample collected in July 2003. The samples belonging to the genus *Calliergon* show similar values, ranging from a value of 18.15‰ (*Calliergon stramienum*) to a value of 18.99‰ (*Calliergon cordifoilum*, August 2004). The average $\delta^{18}O$ value for the moss cellulose of this site is 19.43‰.

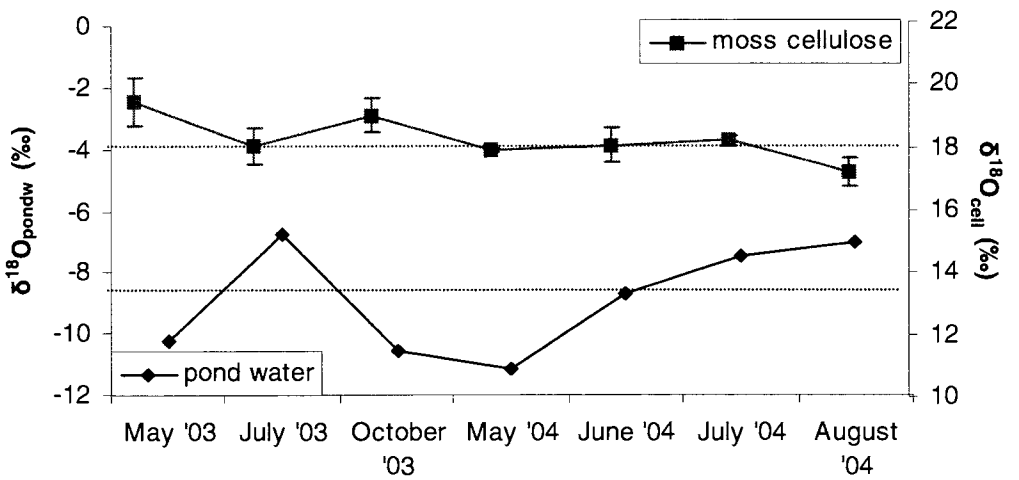

Figure 31. Comparison between the $\delta^{18}O$ values of moss cellulose and pond water for the Au Train samples. The dashed lines indicate the average $\delta^{18}O$ values for pond water (blue line) and cellulose (brown line).

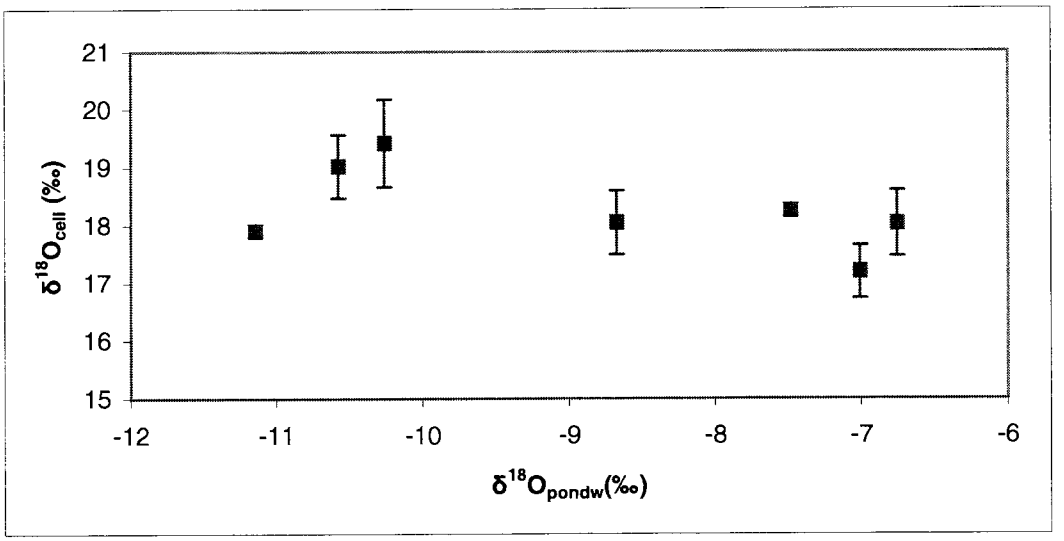

Figure 32. Plot of the Au Train $\delta^{18}O$ values of moss cellulose against the $\delta^{18}O$ values of pond water.

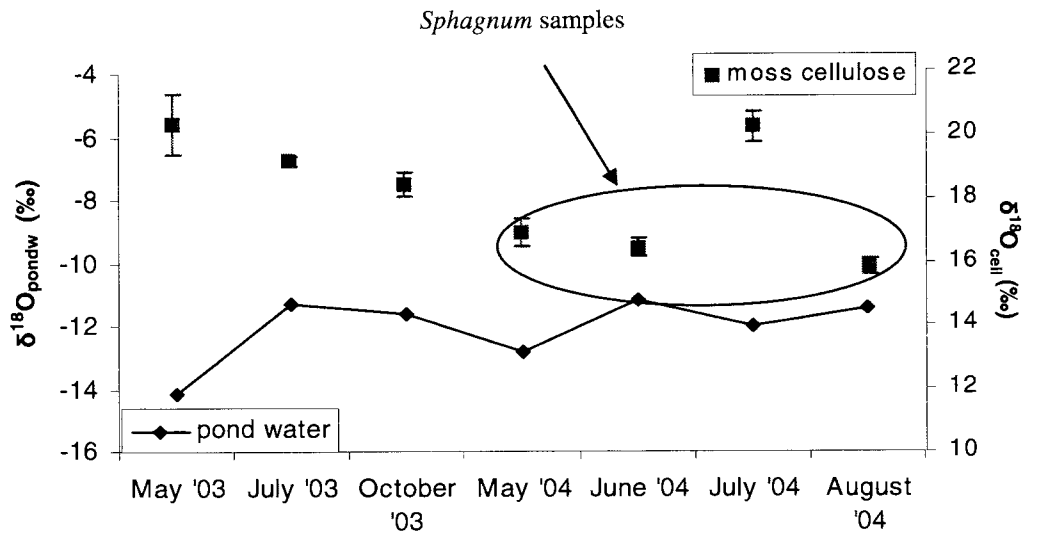

Figure 33. Comparison between $\delta^{18}O$ values of moss cellulose and pond water for the Grand Traverse samples.

DISCUSSION

WATER SAMPLES

The δ-values of groundwater are similar for the three sites, and they are considerably lower than the average isotopic values of rainwater during the growing season. This indicates that aquifer recharge in the north-western Michigan's Upper Peninsula occurs before the beginning of the growing season when appreciable amounts of snowmelt infiltrate, producing low δ-values of water in the saturated zone. This finding is supported by the study of Nicholas et al. (2001) who reported that in Michigan most recharge occurs between the first hard frost in the fall and the beginning of the growing season in the spring.

Another observation common to the three sites is that δ-values of moss water are not statistically different from the values of pond water. This indicates that in general the water surrounding the mosses is isotopically equivalent to pond water. Pendall et al. (2001) also investigated the isotopic composition of moss water in a peat bog located in Patagonia. This study, however, did not provide values of the isotopic composition of bog water. It only showed that, in contrast to terrestrial plants, the isotopic composition of leaf water in aquatic mosses lies close to the meteoric water line and, therefore, is not affected by evaporation. The results reported here show that moss water δ-values are not statistically different from pond water δ-values, which is consistent with the conclusion of Pendall et al. (2001) since the studied ponds are not affected by evaporation.

Another characteristic common to the three sampling localities is the seasonal variability in pond water δ-values. However, a detailed investigation of the values obtained at each

locality shows different causes for this variability, pointing to different hydrological characteristics at the three studied sites.

The seasonal variability of δ-values for pond water samples collected in Au Train (Figures 10, 11) is mainly caused by a shift in the relative importance of meteoric precipitation and groundwater inflow as sources of water for the pond during the growing season. Figures 10 and 11 show that the δ-values recorded for pond water samples are remarkably lower compared to those of rainwater early in the season, and these δ-values tend to approach those of the samples collected later in the growing season (Figures 10, 11). This indicates that the main source of water for this pond is represented by groundwater inflow early in the growing season and that the relative contribution of rainfall increases as the season proceeds. A $\delta^{18}O$-δD plot for water samples shows that the δ-values for pond water plot along a line with a slope similar to that of the LMWL and within the uncertainty associated with the LMWL calculated by Harvey (2001) (Figure 16). This indicates that evaporation is not an important process affecting the water balance of this pond because evaporating water bodies have δ-values that considerably deviate from LMWLs along lines with smaller slopes than those of LMWLs.

The analysis of δ-values of water samples collected in Grand Traverse shows a small seasonal variability in pond water δ-values. Moreover, pond water δ-values are considerably lower than those of rainwater for the entire growing season and are not statistically different from groundwater δ-values (Figures 17, 18). This indicates that the source of water for this pond is mainly represented by groundwater. Rainwater is, in this case, a minor component of the water balance of this pond for most of the growing season and it becomes important only at the end of the season. This is confirmed by the pH measurements of the pond water

samples, which yield an average value of 5.4. This value is typical of a groundwater-dominated peatland (Moore and Bellamy, 1974). Similar to the Au Train pond, evaporation is not an important process for the hydrology of this pond since the δ-values of pond water samples, along with groundwater δ-values, plot close to the LMWL (Figure 23). The minor role of meteoric precipitation as source of water for this pond could be related to canopy effect by trees surrounding this pond. Unlike the Au Train site, this locality is characterized by a thick forest (Figure 7). Leaves of trees can intercept rainfall, preventing significant amounts of raindrops from falling into the pond.

Similar to the previous two sites, pond water δ-values recorded for Ontonagon indicate that evaporation is not an important process affecting the water balance of the pond because the δ-values plot near (within the uncertainty) the LMWL (Figure 30). The hydrology of this site is very similar to that of the Grand Traverse pond. In other words, the peatland in Ontonagon is dominated by groundwater inflow with a contribution of rainwater that becomes important only late in the growing season. The pH measurements of pond water samples, showing an average value of 5.7, are consistent with a groundwater-dominated peatland (Moore and Bellamy, 1974). Similar to the other two studied sites, groundwater samples δ-values plot close to the LMWL, clearly indicating their meteoric origin (Figure 30).

Because of the absence of studies aimed to investigate the hydrology of wetlands in this region, a direct comparison with published data is not possible. However, the study of Sheldock et al. (1993), conducted on wetlands located in southern Michigan, confirms the major role of groundwater for the hydrology of peatlands located between beach ridge complexes of the Great Lakes. Furthermore, studies of wetlands in regions characterized by

continental climate also support a limited evaporative enrichment. For instance, Wolfe and Edwards (1997) reported data of the isotopic composition of water samples collected in the western Taimyr Peninsula, Russia. These authors found that δ-values of lakes and streams from this region plotted close to the LMWL. They explained the minor role of evaporation on the water balance of those lakes and streams as a result of high relative humidity affecting the region during the growing season (e.g., 70-80% during July). Since relative humidity for that area is similar to the growing season average value of the western Michigan's Upper Peninsula (74%), it is then reasonable to expect a limited role of evaporation on the water balance of the ponds in Au Train, Grand Traverse, and Ontonagon.

Similar findings were obtained by Jasinski et al. (1998) for a peatland located in the Lena River valley, Siberia. These authors provided values of the isotopic composition of peat pore water for a core spanning more than 4000 years. The isotope data show no evidence of evaporation for the entire time period represented by the core. The progressive increase in pore water δ-values starting 4750 years B.P. is interpreted as a result of higher input of summer precipitation versus groundwater inflow.

In conclusion, the three investigated wetlands are all characterized by limited evaporative enrichment. The ponds located in Grand Traverse and Ontonagon have a similar hydrology, characterized by the dominance of groundwater inflow on their water balance. The low δ-values of snowmelt determine the overall isotopic composition of pond water and groundwater. The pond located in Au Train, on the other hand, shows higher seasonal variability in water δ-values, and its hydrology is characterized by the interplay of groundwater and meteoric precipitation.

MOSS CELLULOSE

Because of hydrological differences of the studied ponds and the different moss species collected at each sampling location, it is necessary to conduct site-specific discussions of the cellulose δ-values. The detailed investigation of water samples collected in Au Train highlights the seasonal variability of pond water and moss water δ-values caused, as mentioned before, by the relative contribution of groundwater and meteoric precipitation during the growing season. The $\delta^{18}O$ values obtained for moss samples collected at this site indicate that the observed variability of the potential source waters is not fully expressed in cellulose (Figures 31, 32). Whereas $\delta^{18}O$ values of pond water and moss water range by more than 4‰, cellulose δ-values only vary by 2‰. The small variability of cellulose δ-values is in part due to the fact that, in contrast to the other two sampling locations, mosses collected at this site belong to the same species (*Sphagnum cuspidatum*) except for the sample collected in July 2003 corresponding to *Sphagnum centrale*. In addition to that, the collected mosses were completely submerged, thereby eliminating any possible effect of leaf water enrichment process on cellulose δ-values.

Given the constancy in phenology and ecology of the sampled mosses, several hypotheses might explain the lower isotopic variability found in moss cellulose relative to that of surface water. As proposed by Menot-Combes et al. (2002), one reason could involve that the water used by mosses to synthesize cellulose is 20-30 cm below the surface. These authors show that this deeper water source is isotopically more homogeneous than surface water. The data presented here, however, indicate that moss water samples, which represent water contained within hyaline cells of the mosses, are isotopically equivalent to pond water samples. This finding suggests that the water used to synthesize cellulose shows the same

isotopic variability of pond water. Therefore, it is likely to assume that pond water represents the water source for the synthesis of cellulose.

Another hypothesis proposed by the same authors is based on the observation that some mosses considerably reduce photosynthesis or even stop it when their cell water content falls below an optimal value (Williams, 1996). Therefore, according to Menot-Combes et al., the driest period of the growing seasons could not be reflected in moss cellulose δ-values. In the case of the Au Train samples, however, the collected mosses were always growing completely submerged and, therefore, their growth was unlikely to be water-limited. As suggested by Hulme and Blyth (1982) for *Sphagnum* mosses growing in south-west Scotland, growth in *Sphagnum cuspidatum* proceeds without interruptions for the entire growing season under unlimited water source. Therefore, growth limited by water could happen in species growing on hummocks, which can be water-limited during the driest periods of the growing season. The mosses collected in Au Train, however, are all typical hollow species and, therefore, it is reasonable to assume a continual growth period extending throughout the entire growing season.

Based on the data obtained for mosses and moss water samples collected in Au Train, it is possible to conclude that moss cellulose integrates the variations observed in source water over the entire growing season reflecting, in this way, the average isotopic composition of pond water. Several authors have found that cellulose in aquatic plants is 27±3‰ enriched with respect to source water (Epstein et al., 1977; De Niro and Epstein, 1981; Aucour et al., 1996). This biochemical enrichment factor seems to be constant within similar genera and also within similar plant forms (De Niro and Epstein, 1981). The data showed in Table 4 indicate an average enrichment factor of *Sphagnum* cellulose of 27 ± 1‰ with respect to the

average pond water $\delta^{18}O$ value, which is in excellent agreement with the enrichment factor reported in the literature. The analysis of mosses and water samples collected at this site allows for a better understanding of the complex relationship between the isotopic composition of cellulose and that of possible water sources. The abundance of *Sphagnum cuspidatum* mosses in this site, the constancy of the relationship between *Sphagnum* cellulose and source water $\delta^{18}O$ values, and the importance of meteoric precipitation in pond hydrology suggest that Au Train peat $\delta^{18}O$ values can reliably be used for paleoclimate reconstructions of the Great Lakes region.

The analysis of samples collected in Grand Traverse, on the other hand, confirm the conclusion of Menot-Combes et al. (2002), indicating the necessity of conducting paleoclimate reconstructions at the species level. The analysis of moss samples collected in Grand Traverse show statistically significant lower $\delta^{18}O$ values for *Sphagnum cuspidatum* cellulose compared to *Drepanocladus fluitans* and *Aulacomnium palustre* cellulose. Assuming a constant biochemical enrichment factor of 27±3‰ for the two species, two hypotheses can be formulated to explain this difference in $\delta^{18}O$ values. The first hypothesis involves differences in the growing season for the two species. *Sphagnum cuspidatum* could have a longer growing season relative to that of *Drepanocladus fluitans*. Under this scenario, *Sphagnum cuspidatum* would reflect lower $\delta^{18}O$ values of source water typical of the early spring and late autumn. The second hypothesis is related to ecological constraints for the two species. Whereas *Sphagnum cuspidatum* is restricted to a vertical zone ranging from 10 cm below and 5 cm above the water table where the relative humidity is always close to 100% (Vitt et al., 1975), some varieties of *Drepanocladus fluitans* grow on wet rocks but they are not always submerged (Grout, 1903). The studied samples of *Drepanocladus fluitans*,

therefore, could be influenced by leaf water enrichment processes that produce higher cellulose $\delta^{18}O$ values. Based on the data obtained, it is not possible to determine without uncertainty which of these two hypotheses best explain the difference in isotopic composition of *Sphagnum* and *Drepanocladus* cellulose. However, given the limited variability of pond water $\delta^{18}O$ values of this site (Figure 18, Table 2), it is possible to favor the second hypothesis. With the exception of the pond water sample collected in May 2003, the range of pond water $\delta^{18}O$ values of this site is only of 1.6‰. Such a small variability cannot explain the 3.2‰ difference in the average oxygen isotopic composition of the *Sphagnum* and *Drepanocladus* cellulose.

The analysis of samples collected in Ontonagon confirms the higher variability of cellulose $\delta^{18}O$ values when species with different ecology are involved. None of the samples collected in this site is strictly aquatic. Although samples of *Calliergon* were always found to grow almost completely submerged, every species collected at this site can grow at least partially emerged. Because of the effect of leaf water enrichment, the species of this site show an average enrichment factor higher than the species of the other two sites. The sample of *Drepanocladus revolvens*, which was growing completely emerged at the time of sampling, shows the highest enrichment, followed by the terrestrial species *Callicladium haldanianum*. The $\delta^{18}O$ values of *Calliergon* are the lowest for this site and show a small inter-sample variability. The two *Sphagnum squarrosum* samples, on the other hand, show a difference of 2‰ in their $\delta^{18}O$ values. This difference is due to the fact that the sample collected in August 2004 was growing emerged and far from the water table, whereas the sample collected in October 2003 was growing partially submerged.

The analysis of moss samples collected in Ontonagon, in addition to confirm the importance of conducting peat-based paleoclimate reconstructions at the species level, allow for the identification of the species that are more useful for paleoclimate reconstructions. Because of the variability in its ecology, which is reflected in different cellulose $\delta^{18}O$ values between samples of the same species, it is not recommended to use *Sphagnum squarrosum* cellulose for paleoclimate reconstructions. On the other hand, the genus *Calliergon* seems to have a well-constrained ecology, showing therefore a constant relationship between the $\delta^{18}O$ values of its cellulose and that of pond water.

Based on the measured cellulose and water isotope values, it can be concluded that paleoclimate interpretations based on peat cellulose $\delta^{18}O$ values must take into account both hydrology and moss species distribution. The Au Train site seems to be the most suitable for paleoclimatic studies because of the abundance of *Sphagnum cuspidatum* which, having a constrained ecology, shows a constant relationship between cellulose and pond water $\delta^{18}O$ values. In this case, $\delta^{18}O$ values of *Sphagnum cuspidatum*-peat cellulose would allow for the calculation of the growing season average isotopic composition of pond water with an uncertainty of only 1‰ after the application of a 27‰ enrichment factor. Isotope values of rainwater could then be inferred once local hydrological conditions are taken into consideration. The main assumption of this approach is that growth for the mosses forming peat deposits has never been water-limited, thereby allowing mosses to grow continuously throughout the entire growing season.

Isotope results for Grand Traverse and Ontonagon highlights the necessity of performing species-specific studies on mosses for paleoclimatic investigations. Because of different ecological constraints, the relationship between cellulose and pond water $\delta^{18}O$ values is

variable. Therefore, the application of the 27±3‰ enrichment factor for the interpretation of bulk peat $\delta^{18}O$ values can lead to erroneous reconstructions of pond water and, thereby, of rainwater isotope values. On the other hand, the selection of *Sphagnum cuspidatum*-peat cellulose would allow a correct reconstruction of the growing season average pond water $\delta^{18}O$ values which would likely reflect the isotopic composition of late fall-early spring meteoric precipitation.

CONCLUSIONS

The main purpose of this study was to analyze the isotopic composition of modern mosses and that of their possible water sources to calibrate reliable plant-climate relationships, which can be applied to the interpretation of peat isotope data in future studies. The investigation of pond water samples highlighted the effect of hydrology on the three investigated peatlands. Whereas rainwater and groundwater inflow controls the hydrology of the Au Train pond, groundwater inflow dominates the water balance in the Grand Traverse and Ontonagon ponds. Isotope data indicate a negligible effect of evaporation on the overall hydrological balance of the studied ponds.

Moss samples collected in Au Train show a small variability in cellulose $\delta^{18}O$ values relative to pond water $\delta^{18}O$ values that likely resulted from continuous growth of submerged *Sphagnum* mosses throughout the growing season. Cellulose from *Sphagnum cuspidatum* is constantly enriched in ^{18}O by 27±1‰ relative to the average isotopic composition of pond water, suggesting a reliable application of this species in future paleoclimatic studies.

The $\delta^{18}O$ values of mosses collected in Grand Traverse and Ontonagon show a high variability and a non-constant relationship with the isotopic composition of pond water, pointing to the necessity of conducting peat-based paleoclimatic studies at the species level. This high variability was due to the different ecological constraints for the moss species or to different length of their growing season.

Results from this study indicate the need for understanding the effect of local hydrology and the ecological constraints of mosses in peat-based paleoclimate reconstructions.

REFERENCES

Ackerman, E.A., 1941, The Koppen classification of climates in North America. *Geographical Review* **31**, 105-111.

Aravena, R., and Warner, R.G., 1992, Oxygen-18 composition of *Sphagnum*, and microenvironmental water relations. *Bryologist* **95**, 445-448.

Argyilan E.P., and Forman, S.L., 2003, Lake level response to seasonal climatic variability in the Lake Michigan-Huron system from 1920-1995. *Journal of Great Lakes Research* **29**, 488-500.

Aucour, A.M., Hillaire-Marcel, C., and Bonnefille, R., 1996, Oxygen isotopes in cellulose from modern and Quaternary intertropical peatbogs: implications for paleohydrology. *Chemical Geology* **129**, 341-359.

Baedke, S.J., and Thompson, T.A., 2000, A 4,700-year record of lake level and isostasy for Lake Michigan. *Journal of Great Lakes Research* **26**, 416-426.

Balco, G., Belknap, D.F., and Kelley, J.T., 1997. Paleoclimatic implications of Holocene lake-level fluctuations, Owasco Lake, New York: Comment. *Geology* **25**, p. 383.

Beuning, K.R.M., Kelts, K., Ito, E., and Johnson, T.C., 1997, Paleohydrology of Lake Victoria, East Africa, inferred from $^{18}O/^{16}O$ ratios in sediment cellulose. *Geology* **25**, 1083-1086.

Booth, R.K., Jackson, S.T., and Thompson, T.A., 2002, Paleoecology of a northern Michigan lake and the relationship among climate, vegetation, and Great Lakes water levels. *Quaternary Research* **57**, 120-130.

Bowen, G, 2003, The online isotopes in precipitation calculator. http://es.ucsc.edu/%7egbowen/form.html (Retrieved: 10/18/2004)

Bradley, R.S., 1999, Paleoclimatology. Harcourt Academic Press, Burlington MA. 613 pp.

Brenninkmeijer, C.A.M., van Geel, B., and Mook, W.G., 1982, Variations in the D/H and $^{18}O/^{16}O$ ratios in cellulose extracted from a peat bog core. *Earth and Planetary Science Letters* **61**, 283-290.

Bryson, R.A. and Hare, F.K., 1974, The climates of North America. *In*: R.A. Bryson and F.K. Hare (Ed.), Climates of North America, World Survey of Climatology, 11. Elsevier, Oxford, 1-47.

Cohen, S.J., 1986, Impact of CO_2 induced climatic change on water resources in the Great Lakes Basin. *Climatic Change* **8**, 135-153.

Coleman, M.L., Shepherd, T.J., Durham, J.J., Rouse, J.E., and Moore, G.R., 1982, Reduction of water with zinc for hydrogen isotope analysis. *Analytical Chemistry* **54**, 993-995.

Craig, H., 1961, Isotopic variations in meteoric waters. *Science* **133**, 1702-1708.

Craig, H., and Gordon, L.I., 1965, Deuterium and oxygen-18 isotope variations in the ocean and marine atmosphere. Stable Isotopes in Oceanography Studies and Paleotemperatures. Consiglio Nazionale delle Ricerche, Laboratorio di Geologia Nucleare, Pisa, 222pp.

Croley, T.E.II, Quinn, F.H., Kunkel, K.E., and Changnon, S.A., 1996, Climate transposition effects on the Great Lakes hydrological cycle. NOAA Technical Memorandum ERL GLERL-89, Great Lakes Environmental Research Laboratory, Ann Arbor, MI, 100 pp.

Crum, H., 1973, Mosses of the Great Lakes forest. *In*: Contributions from the University of Michigan Herbarium, **10**, 1-404. University Herbarium, University of Michigan Ann Arbor.

Crum, H., 1988, A focus on peatlands and peat mosses. University of Michigan Ann Arbour. 306 pp.

Dalrymple, R.W., and Carey, J.S., 1990, Water-level fluctuations in Lake Ontario over the last 4000 years as recorded in the Cataraqui River lagoon, Kingston, Ontario. *Canadian Journal of Geological Science* **27**, 1330-1338.

Dansgaard, W., 1964, Stable isotopes in precipitation. *Tellus* **16**, 436-468.

Davis, M., Douglas, C., Calcote, R., Cole, K. L.,Winkler,M. G., and Flakne, R., 2000, Holocene climate in the western Great Lakes National Parks and Lakeshores: Implications for future climate change. *Conservation Biology* **14,** 968–983.

Delcourt, P.A., Nester, P.L., Delcourt, H.R., Mora, C.I., and Orvis, K.H., 2002, Holocene lake-effect precipitation in Northern Michigan. *Quaternary Research* **57**, 225-233.

DeNiro, M.J., and Epstein, S., 1981, Isotopic composition of cellulose from aquatic organism. *Geochimica et Cosmochimica Acta* **45**, 1885-1894.

Dorr, J.A. Jr, and Eschman, D.F., 1970, Geology of Michigan. The University of Michigan Press, Ann Arbor. 476 pp.

Edwards, T.W.D., Wolfe, B.B., and MacDonald, G.M., 1996, Influence of changing atmospheric circulation on precipitation $\delta^{18}O$-temperature relations in Canada during the Holocene. *Quaternary Research* **46**, 211-218.

Edwards, T.W.D., Wolfe, B.B., Gibson, J.J., and Hammarlund, D., 2004, Use of water isotope tracers in high-latitude hydrology and paleohydrology. *In*: R. Pienitz, M.S.V. Douglas, and J.P. Smol (Eds.) Long-term Environmental Change in Arctic and Antarctic Lakes. Developments in Paleoenvironmental Research. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Ehleringer, J.R., and Monson, R.K., 1993, Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* **24**, 411-439

Eichenlaub, V.L., 1979, Weather and climate of the Great Lakes Region. University of Notre Dame Press, South Bend, IN. 335 pp.

Epstein, S., and Mayeda, T., 1953, Variation of ^{18}O content of water from natural sources. *Geochimica et Cosmochimica Acta* **42**, 213-224.

Epstein, S., Thompson, P., and Yapp, C.Y., 1977, Oxygen and hydrogen isotopic ratios in plant cellulose. *Science* **198**, 1209-1215.

Farquhar, G.D., Barbour, M.M., and Henry, B.K., 1998, Oxygen isotope composition of leaf material. *In*: H. Griffiths (Ed.), Stable Isotopes: Integration of Biological, Ecological and Geochemical Processes, BIOS Scientific Publishers, Oxford, 1998, pp. 27–48.

Ferrand W.R., and Drexler C.W., 1985, Late Wisconsian and Holocene history of the Lake Superior basin. *In*: P.F. Karrow and P.E. Calkin (Eds.) Quaternary Evolution of the Great Lakes. *Geological Association of Canada Special Paper* **30**, 17-32.

Flanagan, L.B., Comstock, J.P., and Ehleringer, J.R., 1991, Comparison of modeled and observed environmental influences on the stable oxygen and hydrogen isotope composition of leaf water in *Phaseolas vulgaris L.. Plant Physiol*ogy **96**, 588-596.

Fraser, G.S., Larsen, C.E., and Hester, N.C., 1990, Climatic control of lake levels in the Lake Michigan and Lake Huron basins. *In*: A.F. Schneider and G.S. Fraser (Eds.) Lake Quaternary History of the Lake Michigan Basin. *Geological Society of America Special Paper* **251**, 75-89.

Gat J.R., and Gonfiantini R., 1981, Stable Isotope Hydrology: Deuterium and Oxygen-18 in the Water Cycle. IAEA Technical Report Series No. 210. IAEA, Vienna, 335pp.

Gonfiantini, R., 1986, Environmental isotopes in lake studies. *In*: Fritz, P. & J.Ch. Fontes (Eds.), Handbook of Environmental Isotope Geochemistry. Volume 2. The Terrestrial Environment, B. Elsevier, Amsterdam, 112-168

Green, J.W., 1963, Wood cellulose. *In*: R.L. Whistler (Ed). Methods in Carbohydrate Chemistry, volume III, Academic Press, New York, 9-20.

Grout, A.J., 1903, Mosses with hand-lens and microscope: a non technical hand-book of the more common mosses of the northeastern United States. Grout, New York, 416 pp.

Hallingback, T. and Hodgetts, N. (compilers), 2000, Mosses, liverworths, and hornworths. Status survey and conservation action plan for bryophytes. IUCN/SSC bryophite specialist group. IUCN, Gland, Switzerland and Cambridge, UK, 106 pp.

Hammarlund D., Barnekow L., Birkis H.J.B., Buchardt B., and Edwards T.W.D., 2002, Holocene changes in atmospheric circulation recorded in the oxygen-isotope stratigraphy of lacustrine carbonates from northern Sweden. *The Holocene* **12**, 355-367.

Harvey, F.E., 2001, Use of NADP archive samples to determine the isotopic composition of precipitation: characterizing the meteoric input function for use in ground water studies. *Ground Water* **39**, 380-390.

Hulme, P.D., Blyth A.W., 1982, The annual growth period of some *Sphagnum* species on the Silver Flowe National Nature Reserve, south-west Scotland. *Journal of Bryology* **12**, 287-291

Jasinski, J.P.P., Warner, B.G., Andreev, A.A., Aravena, R., Gilbert, S.S., Zeeeb,B.A., Smol, J.P., and Velichko, A.A., 1998, Holocene environmental history of a peatland in the Lena River valley, Siberia. *Canadian Journal of Earth Sciences* **35**, 637-648.

Johnson, S.A., 1983, Mosses. Lerner Publication company, Minneapolis, 48 pp.

Johnston, J.W., Baedke, S.J., Booth, R.K., Thompson, T.A., and Wilcox, D.A., 2003, Late-Holocene lake-level variation in southeastern Lake Superior: Tahquamenon Bay, Michigan. *Journal of Great Lakes Research* (in press).

Keddy, P. A., and Reznicek, A. A., 1986, Great Lakes vegetation dynamics: the role of fluctuating water levels and buried seed. *Journal of Great Lakes Research* **12**, 25-36.

Larsen, C.E., 1994, Beach ridges as monitors of isostatic uplift in the upper Great Lakes. *Journal of Great Lakes Research* **20**, 108-134.

Lofgren, B.M., Quinn, F.H., Clites, A.H., Assel, R.A., Eberhardt, A.J., and Luukkonen, C.L., 2002, Evaluation of potential impacts on Great Lakes water resources based on climate scenarios of two GCMs. *Journal of Great Lakes Research* **28**,537-554.

MacDonald, G.M., Velichko, A.A., Kremenetski, C.V., Borisova, O.K., Goleva, A.A., Andreev, A.A., Cwynar, L.C., Riding, R.T., Forman, S.L., Edwards, T.W.D., Aravena, R., Hammarlund, D., Szeicz, J.M., and Gatullin, V., 2000, Holocene treeline history and climate across northern Eurasia. *Quaternary Research* **53**, 302-311.

Maric R., 2003, Coupled isotope-mass balance studies in tundra lakes, Lac de Gras area, N.W.T., Canada. M.Sc. Thesis, University of Waterloo, 145 pp.

McQueen, C.B., 1990, Field guide to the peat mosses of boreal North America. The University Press of New England, 138 pp.

Menot-Combes, G., Burns, S.J., and Leuenberger, M., 2002, Variations of $^{18}O/^{16}O$ in plants from temperate peat bogs (Switzerland); implications for paleoclimatic studies. *Earth and Planetary Science Letters* **202**, 419-434.

Moore, P.D., and Bellamy, D.J., 1974, Peatlands. Springer-Verlag, New York. 221 pp.

Mook W. G., and de Vries J.J., 2000, Environmental isotopes in the hydrological cycle principles and applications. IAEA, Vienna, 280 pp. http://www.iaea.or.at/programmes/ripc/ih/volumes/volume1.htm (Retrieved: 10/18/2004)

Mortsch, L.D., and Quinn, F.H., 1996, Climate scenarios for Great Lakes basin ecosystem studies. *Limnology and Oceanography* **41**, 903-911.

Nicholas, J.R., Blumer, S.P., and McGowan, 2001, Comparison of Hydrologic Data from Monroe County, Michigan, 1991-2001. USGS open file report 2001-498.

Pendall, E., Markgraf, V., White, J.W.C., and Dreier, M., 2001, Multiproxy record of Late Pleistocene-Holocene climate and vegetation changes from a peat bog in Patagonia. *Quaternary Research* **55**, 168-178.

Proctor, M.C.F., Raven, J.A., and Rice, S.K., 1992, Stable carbon isotope discrimination measurements in *Sphagnum* and other bryophytes: physiological and ecological implications. *Journal of Bryology* **17**, 193-202.

Quinn, F.H., 1988, Great Lakes water levels, past, present, and future. *In*: D.H. Hickcox, (Ed) Proceedings of the Symposium on: The Great Lakes: Living with North America's inland waters. American Water Resources Association Technical Publication Series, 83-92

Quinn, F.H., 2002, Secular changes in Great Lakes water seasonal cycles. *Journal of Great Lakes Research* **28**, 451-465

Schimmelmann A., and DeNiro M.J., 1993, Preparation of organic and water hydrogen for stable isotope analysis: effects due to reaction vessels and zinc reagent. *Analytical Chemistry* **65**, 789-792.

Shedlock, R.J., Wilcox, D.A., Thompson, T.A., and Cohen, D.A., 1993, Interactions between ground water and wetlands, southern shore of Lake Michigan, USA. *Journal of Hydrology* **141**, 127-155.

Simpkins, W.W., 1995, Isotopic composition of precipitation in central Iowa. *Journal of Hydrology* **172**, 185-207.

Thompson, T.A., 1992, Beach-ridge development and lake-level variation in southern Lake Michigan. *Sedimentary Geology* **80**, 305-318.

Thompson, T.A., Fraser, G.S., and Olyphant, G., 1988, Establishing the altitude and age of past lake levels in the Great Lakes. Geological Society of America, Abstr. Progr., 20: 392

Vitt, D.H., Crum, H., Snider, A.J., 1975, The vertical zonation of *Sphagnum* species in hummock-hollow complexes in northern Michigan. *The Michigan Botanist* **14**, 190-200.

Waterhouse, J.S., Switsur, V.R., Barker, A.C., Cater, A.H.C., and Robertson, I., 2002, Oxygen and hydrogen isotope ratios in tree rings: how well do models predict observed values? *Earth and Planetary Science Letters* **201**, 421-430.

White, J.W.C., Cook, E.R., Lawrence, J.R., and Broecker, W.S., 1985, The D/H ratios of sap in trees: implications for water sources and tree ring D/H ratios. *Geochimica et Cosmochinica Acta* **49**, 237-246.

Wilcox, D. A., 1989, Responses of selected Great Lakes wetlands to water level fluctuations: Appendix B. *In*: W.D.N. Busch, R. Kavetsky, and G. McCullough (Eds.), Water Level Criteria for Great Lakes Wetlands. International Joint Commission Water Levels Reference Functional Group 2.

Williams, T.G., 1996, Effect of changes in water content on photosynthesis, transpiration and discrimination against $^{13}CO_2$ and $C^{18}O^{16}O$ in *Pleurozium* and *Sphagnum*. *Oecologia* **108**, 38-46.

Wolfe, B.B., and Edwards, T.W.D., 1997, Hydrologic control on the oxygen-isotope relation between sediment cellulose and lake water, western Taimyr Peninsula, Russia:

implications for the use of surface-sediment calibrations in paleolimnology. *Journal of Paleolimnology* **18**, 283-291.

Wolfe, B.B., Edwards, T.W.D., Aravena, R., Forman, S.L., Warner, B.G., Velichko, A.A., and MacDonald, G.M., 2000, Holocene paleohydrology and paleoclimate at treeline, North-Central Russia, inferred from oxygen isotope records in lake sediment cellulose. *Quaternary Research* **53**, 319-329.

Wolfe, B.B., Edwards, T.W.D., Beuning, K.R.M., and Elgood, R.J., 2001, Carbon and oxygen isotope analysis of lake sediment cellulose: methods and applications. *In*: W.M. Last and J.P. Smol (Eds). Tracking Environmental Change Using Lake Sediments: Physical and Chemical Techniques. Developments in Paleoenvironmental Research, Vol. 2. Kluwer Academic Publishers, Dordrecht, The Netherlands, 373-400.

Yakir, D., and DeNiro, M.J., 1990, Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba L. Plant Physiology* **93**, 325–332.

Yakir, D., 1992, Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. *Plant Cell Environment* **15**, 1005–1020.

Yu, Z., McAndrews, J.H., and Eicher, U., 1997, Middle Holocene dry climate caused by change in atmospheric circulation patterns: evidence from lake levels and stable isotopes. *Geology* **25**, 251-254.

Zimmermann, U., Enhalt, D., and Munnich, K.O., 1967, Soil water movement and transpiration: changes in the isotopic composition of water. *In*: Isotopes in Hydrology, International Atomic Energy Agency, Vienna, pp.567-685.